Official Protocol Title:	A Phase I/II, Multicenter, Open-label, Clinical Trial of Intratumoral/Intralesional Administration of RGT100 in Subjects with Advanced or Recurrent Tumors
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CLINICAL STUDY PROTOCOL

A Phase I/II, Multicenter, Open-label, Clinical Trial of Intratumoral/Intralesional Administration of RGT100 in Subjects with Advanced or Recurrent Tumors

Short title: A Phase I/II trial of intralesional administration of RGT100

Protocol Number:	RGT100-001
EudraCT Number:	2016-003028-22
INC Research Study Number:	1007463
Investigational Product:	RGT100-PEI (a RIG-I-Agonist)
Phase:	Phase I/II
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Original Protocol Date: Amendment 1.1 (UK-specific) **Amendment 2.0:**	16 Sept 2016, v1.0 03 Nov 2016 **16 Nov 2016**

1 PROTOCOL APPROVAL SIGNATURES

Protocol Title: A Phase I/II, Multicenter, Open-label, Clinical Trial of Intratumoral/Intralesional Administration of RGT100 in Subjects with Advanced or Recurrent Tumors

Protocol Number: RGT100-001

This study will be conducted in compliance with the clinical study protocol (and amendments), International Council for Harmonisation (ICH) guidelines for current Good Clinical Practice (GCP), and applicable regulatory requirements.

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3 SYNOPSIS

Protocol Number	RGT100-001
Title	A Phase I/II, Multicenter, Open-label, Clinical Trial of Intratumoral/Intralesional Administration of RGT100 in Subjects With Advanced or Recurrent Tumors
Investigational Product	RGT100-PEI (a RIG-I-Agonist)
Study Center	Multicenter
Phase	Phase I/II
Objectives	The primary objectives of the study are to: • Evaluate the safety and tolerability of intratumoral/intralesional injections of RGT100-PEI • Determine the recommended Phase 2 dose feasible for intratumoral/intralesional injections of RGT100-PEI The secondary objective of the study is to: • Explore preliminary anti-tumor activity of RGT100-PEI on injected and un-injected tumor lesions The exploratory objectives of the study are to: • Investigate the type of cytokine release in plasma upon treatment • Investigate the pharmacokinetics (PK) of RGT100-PEI in subjects with advanced or recurrent tumors • Explore potential predictive biomarkers of tumor responses upon intratumoral/intralesional RGT100-PEI therapy* • Evaluate the impact of RGT100-PEI therapy on immune infiltration of injected tumors* *These exploratory objectives will be evaluated in a separate sub-study. Results of these exploratory investigations will not be presented as part of the main Clinical Study Report.
Study Design	This is a Phase I/II multicenter, first-in-human open-label, dose escalation study to evaluate the safety, tolerability, and anti-tumor activity of intratumoral/intralesional injections of RGT100-PEI in subjects with selected advanced or recurrent tumors. The study will be conducted in 2 groups • **Group A:** subjects with transdermally/transmucosally injectable tumors • **Group B:** subjects with injectable liver tumors or liver metastases The study will be conducted in 2 phases for each group • **Dose escalation phase:** All types of tumors accessible for intratumoral/intralesional injection • **Expansion phase:** Within up to 4 different tumor indications to be specified based on preliminary safety and efficacy data as well as feasibility derived from the dose escalation phase.

	Disease evaluation will be performed by magnetic resonance imaging (MRI)/computerized tomography (CT) scan/positron emission tomography (PET) scan. Skin lesions will be measured via photography and calipers. A 3+3 design will be used for dose escalation. For each dose level (DL), the first subject treated will begin treatment 1 week before the next subjects of the group are included in order to allow the assessment of initial safety data.
Number of Subjects	**Dose escalation phase**: Approximately 9 to 24 subjects will be treated in each group during the dose escalation. **Expansion phase**: An additional 15 subjects each in up to 4 different tumor indications (up to 60 subjects in total)

Treatment	Treatment plan: • **Group A:** RGT100-PEI will be injected intratumorally/intralesionally twice a week over a period of 4 weeks • **Group B:** RGT100-PEI will be injected into liver lesions once a week over a period of 4 weeks RGT100-PEI will be at a fixed concentration of 0.2 mg/mL and dose escalation will be based on the total volume of RGT100-PEI injected per subject. The planned DLs to be investigated in this phase of the study are: • DL1: 0.2 mg = 1 mL • DL2: 0.4 mg = 2 mL • DL3: 0.6 mg = 3 mL • DL4: 0.8 mg = 4 mL* *Dose escalation may continue if deemed appropriate by the Safety Review Committee. The corresponding next dose levels beyond the above and the supporting safety data to continue will be described within a substantial amendment to this protocol which will only be implemented once approval has been received. One index lesion will be selected by the Investigator and all injections will be given into this index lesion if possible. All lesions will be injected either directly or under image guided control (e.g. ultrasound). At each injection, the operator should collect the following information: • Location of the lesion in the body • Size of the injected lesion • National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) grading inflammation at the site and time of injection, if any, before and after injection • Type of lesion injected (skin, lymph node, liver) • Planned volume to be injected in the lesion (for eventual intent-to-treat analysis) • Actual volume injected in the lesion (for eventual per dose received analysis) If all lesions undergo complete remission before the end of the 4 weeks therapy, the subjects will stop the treatment until the first disease evaluation (MRI/CT scan/PET scan at 8 weeks). Subjects with partial response or stable disease at the first evaluation at 8 weeks will be followed for a further 4 weeks for another disease evaluation. Subjects with progressive disease will stop the study at Week 8.
Study Duration	Per subject: screening period of up to 3 weeks, treatment period of 4 weeks. The last day is a 30- or 60-day follow-up visit of the last injection. Further non-interventional survival follow-up will be performed every 3 months for a period of 24 months after the last subject has completed the trial.
End of Study	The end of the study will be defined as the point when the last subject has completed the last study follow-up visit (60 days). Survival follow-up after this timepoint will occur but not as part of the study.

Study Population	**Inclusion Criteria** A subject will be eligible for inclusion in this study if all of the following criteria apply: 1. Male or female aged ≥ 18 years 2. Subjects with histologically or cytologically confirmed diagnosis of advanced or recurrent tumors (including lymphomas) for whom all standard treatments have been used or are not feasible or RGT100-PEI is a suitable treatment option and: a. For Group A: has cutaneous, subcutaneous, or lymph node injectable tumors b. For Group B: has injectable liver tumors or liver metastases 3. Eastern Cooperative Oncology Group (ECOG) performance status 0-2 4. Life expectancy > 3 months as assessed by the Investigator 5. Adequate organ function: a. Bone marrow function: Hemoglobin ≥ 10 g/dL (pre-treatment transfusion allowed); lymphocyte count ≥ 0.5×10^9/L; absolute neutrophil count ≥ 0.5×10^9/L; platelet count ≥ 75×10^9/L b. Hepatic function: aspartate transaminase (AST) and alanine transaminase (ALT) ≤ 2 × upper limit of normal (ULN) (3 × ULN in the case of liver metastases); bilirubin ≤ 1.5 × ULN (2 × ULN in case of liver metastases) c. Renal function: creatinine < 1.5 × ULN and/or creatinine clearance ≥ 50 mL/min (Cockroft and Gault) 6. Negative serum pregnancy test within 2 weeks before first dose of study drug if the subject is a woman of childbearing potential. Subjects and subjects' partners of childbearing potential must agree to use birth control consistently and correctly during the study and for at least 6 months after the last study drug application. 7. At least 1 measurable lesion per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 and 1 separate injectable lesion with diameter ≥ 1 cm but < 7 cm 8. Ability to provide written informed consent before any study drug-related screening procedures being performed

Study Population	**Exclusion Criteria** A subject will not be eligible for the trial if any of the following apply: 1. Any tumor-directed therapy within 4 weeks before study treatment 2. Treatment with investigational drugs within 4 weeks before study enrolment 3. Systemic steroids at a dose of > 10 mg of prednisolone, > 2 mg of dexamethasone a day or equivalent, except topical (inhaled, topical, nasal) for the last 28 days and ongoing 4. Subjects with rapidly progressing disease (as determined by the Investigator) 5. Ongoing immune-related adverse events (irAEs) and/or AEs ≥ grade 2 not resolved from previous therapies except vitiligo, stable neuropathy grade 2, hair loss, and stable endocrinopathies with substitutive hormone therapy. 6. Within 4 weeks of major surgery 7. Prior splenectomy 8. Documented history of active autoimmune disorders requiring systemic immunosuppressive therapy 9. Primary or secondary immune deficiency 10. Active allergy requiring systemic medication or active infections requiring anti-infectious therapy 11. Seropositive (except after vaccination) for human immunodeficiency virus (HIV), hepatitis B virus (HBV), or hepatitis C virus (HCV) 12. Clinically significant cardiac disease including heart failure (New York Heart Association, Class III or IV), pre-existing arrhythmia, uncontrolled angina pectoris, or myocardial infarction within 1 year before study entry 13. Dementia or altered mental status that would prohibit informed consent 14. Other severe, acute, or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study assessed by the Investigator 15. History of stroke, seizures, encephalitis, or multiple sclerosis 16. Gastric ulcer or inflammatory bowel disease or Crohn's disease or ulcerative colitis in the last 6 months. 17. Active drug or alcohol abuse 18. Pregnant or breast feeding

Study Parameters	**Safety and tolerability:** The safety and tolerability of intratumoral/intralesional injections of RGT100-PEI will be evaluated by the incidence of AEs (all AEs will be evaluated according to NCI-CTCAE version 4.03), serious adverse events (SAEs), dose-limiting toxicities (DLTs), and use of concomitant medications. Safety assessments will include: electrocardiograms (ECGs), physical examinations, ECOG performance status, vital signs and clinical laboratory samples (hematology, biochemistry, cytokines and urinalysis). **Anti-tumor activity:** To explore the anti-tumor activity of RGT100-PEI on injected and un-injected tumor lesions will be evaluated with the following assessments: Tumor assessments, imaging, tumor biopsies to evaluate immunogenic cell death and immune infiltration of injected tumors. Tumor response rate will be evaluated radiologically using immune-related RECIST criteria (irRECIST). **Pharmacokinetic:** The PK of RGT100-PEI will be measured from blood samples collected at the start and end of the study. Standard PK parameters will be determined from the RGT100-PEI concentration in plasma.
Statistical Analysis	Descriptive and inferential statistics will be used to summarize the data. Continuous variables will be summarized by number, mean, standard deviation, median, minimum and maximum values. Categorical variables will be summarized by subject counts and related percentages. Two subject populations will be used for the analyses. The safety population will consist of all subjects who received at least 1 dose of RGT100-PEI. The evaluable population will consist of all enrolled subjects who complete Week 4 or withdraw early for experiencing a DLT. Safety will be assessed on the basis of AE reports, clinical laboratory data, vital signs, body weight, ECG, physical examinations and ECOG performance status. All safety summaries will be presented by total and dose level (DL) for the dose escalation phase and total and group (tumor-specific group) for the expansion phase. The number of subjects experiencing each AE will be summarized by the Medical Dictionary for Regulatory Activities (MedDRA) system organ class, MedDRA preferred term, NCI CTCAE grade and relationship to study treatment. Serious adverse event and irAEs will be summarized separately. Summary statistics (mean, median, standard deviation and range as appropriate) by cohort, group and overall will be presented for vital signs, physical examination findings, ECG parameters and clinical laboratory tests (clinical chemistry, hematology, cytokines and urinalysis) with changes from baseline also presented. For laboratory data, abnormal values will be flagged in the data listings. All safety data will be included in the subject data listings. On an exploratory basis, common statistical methodology for response rates and survival time analysis may be applied. Counts and percentages, with their corresponding exact 95% confidence interval, will be calculated for the binomial endpoints (i.e., response rate). Logistic regression analyses may be used to test potential predictive factors for overall response.

	Plasma concentrations of RGT100-PEI will be summarized by cohort and group using descriptive statistics (number, geometric mean, arithmetic mean, standard deviation and coefficient of variation [CV], minimum, maximum) at each timepoint.

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5 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	adverse event
ALT	alanine transaminase
AST	aspartate transaminase
CR	complete response
eCRF	electronic case report form
CRO	contract research organization
CT	computerized tomography
DL	dose level
DLT	dose-limiting toxicity
DR	duration of response
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HBV	hepatitis B virus
HCV	hepatitis C virus
HED	human equivalent dose
HIV	human immunodeficiency virus
IB	investigator brochure
ICH	International Council for Harmonisation
ir	immune-related
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
MFD	maximum feasible dose
MTD	maximum tolerated dose
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NE	not evaluable
NOAEL	no observed adverse effect levels
PD	progressive disease
PD-1	programmed cell death protein 1

PD-L1	programmed cell death ligand 1
PET	positron emission tomography
PK	pharmacokinetic
PR	partial response
REC	Research Ethics Committee
RECIST	Response Evaluation Criteria in Solid Tumors
RIG-I	retinoic acid–inducible gene I
RP2D	recommended Phase 2 dose
SAE	serious adverse event
SC	subcutaneous
SD	stable disease
SOP	standard operating procedure
SRC	Safety Review Committee
SUSAR	suspected unexpected serious adverse reaction
TMTB	total measured tumor burden
ULN	upper limit of normal

6 INTRODUCTION

6.1 Background

The World Health Organization reports that 8.2 million people die each year from cancer, an estimated 13% of all deaths worldwide.[1] These numbers highlight the need for ongoing research in this area. The mainstays of cancer treatment commence with early detection, accurate diagnoses, and a multimodal treatment approach (surgery, chemotherapy, and radiation therapy). More recently, molecular-targeted therapies and immunotherapies have emerged as treatment options. There are several types of immunotherapy, including monoclonal antibodies, non-specific immunotherapies, and cancer vaccines.

In the past few years significant progress has been made in the field of immunotherapy. In particular antibodies targeting immune checkpoints have yielded impressive improvements in clinical outcomes for a range of tumor types. Results have led to the approvals of nivolumab and pembrolizumab, targeting programmed death receptor 1 (PD-1) in melanoma and non-small-cell lung cancer. Approvals for these and other PD-1 or programmed death ligand 1 (PD-L1)-targeted antibodies have also been granted in Hodgkin's lymphoma, renal cell cancer, and urothelial cancer, with additional trial results in other indications eagerly anticipated. Ipilimumab, targeting cytotoxic T-lymphocyte antigen 4, has been approved for use in melanoma, including as an adjuvant therapy, and the combination of nivolumab and ipilimumab has demonstrated superior response rates to either drug alone in this indication. Interest in this approach has also been stimulated by the impressive durability of some responses to immunotherapy, which can in some cases be measured in years.

Despite these unprecedented advances the majority of advanced cancer patients do not respond to immunotherapy, due to local immune tolerance at the tumor, absence of effect or cells, or the development of resistance through a variety of adaptive mechanisms. There is therefore considerable interest in the development of combination strategies to overcome these hurdles to successful treatment. Promising results have been seen with the combinations of talimogene laherparepvec, indolamine-2,3-dioxygenase (IDO) inhibitors, or radiotherapy and checkpoint inhibitors but definitive clinical data are not yet available. There remains considerable scope to improve upon current therapy, even if combinations under development prove more effective than current standards of care.

Rigontec has developed RGT100, a cytosolic receptor molecule for intratumoral/intralesional injection. RGT100 injections result in subsequent retinoic acid–inducible gene I (RIG-I) activation, which has been shown to induce a potent innate as well as adaptive immune response and is being investigated in this study to treat subjects with advanced or recurrent tumors.

6.2 Retinoic Acid–inducible Gene I

6.2.1 Apoptosis Induction and Immune Stimulation after Retinoic Acid–inducible Gene I Activation

Retinoic acid–inducible gene I is a cytosolic receptor molecule that was initially discovered as a sensor for viral RNAs. In nonclinical experiments ligand-induced activation of RIG-I in tumor cells leads to induction of a potent innate and adaptive immune response against the tumor. This effect is accompanied by the induction of apoptosis, which is prominent for tumor cells. In combination both effects lead to massive destruction of the treated tumor,

including the untreated metastases. Antigens released during immunogenic apoptosis serve as broad source of antigens to develop a strong anti-tumor response. In healthy cells, stimulation of RIG-I leads to a secretion of type I interferons, but in physiological doses not to apoptosis.[2] This serves as a strong adjuvant effect to support a highly efficient systemic anti-tumor immune response. Combining both, tumor-born antigens and a strong immune activation by the adjuvant function, tumors turn into their own vaccines without prior restriction to tumor-specific antigens.

6.2.2 Sensitivity of Tumor Cells to Retinoic Acid–inducible Gene Activation

While the expression of many receptors of the innate immune system is restricted to a certain subset of cells (e.g., only in dendritic cells), RIG-I is ubiquitously expressed. This observation is in line with its pivotal role in antiviral defense against RNA viruses, a system that must be functional in virtually all somatic cells. Rigontec's founders demonstrated that RIG-I activation in tumor cell lines triggers cells death, whereas non-malignant cells showed only marginal reduction in cell viability.[3] Subsequent studies reveal that RIG-I stimulation initiates the mitochondrial apoptosis pathway, which is preferentially due to the induction of the pro-apoptotic BH3-only proteins Puma and Noxa in RIG-I–stimulated cells. The same pro-apoptotic signal was also observed in non-malignant cells but these cells were comparatively less sensitive to apoptosis induction than melanoma cells, as the upregulation of endogenous Bcl-x_L rescued primary cells from RIG-I–mediated pro-apoptotic signal. Since Bcl-x_L is dysregulated in most tumor cells it can no longer serve as a rescue mechanism.

6.2.3 Formation of Immune-activating Exosomes

Apart from the direct immune-activating effect of RIG-I agonists in immune cells, the founders were able to show that RIG-I activation in tumor cells besides apoptosis leads to formation of immune-activating exosomes. These exosomes contain the RIG-I agonist and show the natural killer cell receptor ligand BAG6 on the surface. Thereby they trigger a natural killer cell-mediated cytotoxicity as well.

6.2.4 Reprogramming of the Tumor Microenvironment

Several studies revealed that breaking the local dominance of myeloid-derived suppressor cell within the tumor microenvironment is a key step to effective anti-tumor immunity. Interferon α (IFNα), one of the lead cytokines produced after RIG-I activation, can potently induce myeloid-derived suppressor cell differentiation. Thereby they exhibit a significantly reduced T-cell suppressive phenotype. The immunosuppressive tumor tissue is converted into a T-cell–promoting environment facilitating the generation of a T-cell–dependent anti-tumor immune response.

The RIG-I–dependent onco-immunotherapy can be successful due to its multitude of contact points:

- It directly activates immune cells.
- It induces immunogenic cell death in tumor cells and thereby generates a tumor-born antigen pool for adaptive immunity.
- It leads to release of immune-activating exosomes to promote tumor-specific natural killer cell cytotoxicity.

- It reprograms the tumor microenvironment to facilitate the anti-tumoral immune response.

Refer to the Investigator Brochure (IB)[4] for addition details of the RIG-I activation.

6.3 RGT100-PEI

6.3.1 Delivery of RGT100-PEI

Because RIG-I is a cytosolic receptor, various agents have been investigated to efficiently deliver RGT100 into the cytosol. *In vivo*-jetPEI™ (Polyplus Transfection) has been identified as the most efficient delivery agent. A stable formulation procedure to generate stable and define particles for storage has been developed as ready-to-use dispersion. *In vivo*-jetPEI™ has been tested in several clinical studies for the delivery of DNA in gene therapy trials. This linear polyethylenimine agent is regarded as an excipient and has a Drug Master File placed at the United States Food and Drug Administration (FDA) for the use in clinical trials.

Essential parts of the mode of action of RGT100, like apoptosis induction in tumor cells and reprogramming of the tumor microenvironment, are local effects, most efficiently induced by high local concentration of RGT100. Immune activation can be achieved by systemic and local administration. Therefore, for most potent anti-tumoral activity and establishment of a proper adaptive immunity by either local administration or targeted systemic administration is desired.

Due to tissue localization and composition, local administration faces challenges. Local administration is limited by maximum injection volumes feasible in target tumor tissue. The concentration of the RGT100-PEI particles in the injection dispersion is fixed to 0.2 mg/mL to achieve stable complexes. Therefore the maximum dose of RGT100-PEI per tumor is limited by the volume injectable in the tumor tissue.

6.3.2 *In vivo* Proof of Concept Studies of RGT100-PEI

Due to the easy access for intratumoral application, murine melanoma models were chosen for the *in vivo* proof-of-concept studies in mice. In this hard-to-treat spontaneous melanoma model in mice, RGT100-PEI was able to significantly reduce tumor growth and also reduce the number of cutaneous lesions.

Proof-of-concept studies have also been extended to relevant cell-based models for glioblastoma and colon cancer as well as *in vivo* models for malignant melanoma, pancreatic cancer, prostate cancer, ovarian cancer, breast cancer, and hepatocellular carcinoma. So far all tumor models tested have been sensitive to RIG-I–mediated therapy.

Refer to the IB[4] for additional details of *in vivo* studies of RGT100-PEI.

6.3.3 Nonclinical Pharmacokinetic Studies of RGT100-PEI

Initial *in vitro* stability assays of uncomplexed and complexed RGT100 duplex showed that uncomplexed RGT100 exhibited an elimination half-life of 2 to 4 hours (plasma/serum). Complexed RGT100, however, was stable for over 48 hours *in vitro* in plasma and serum. Therefore, it was concluded that, once decomplexed, RGT100 is rapidly degraded within the human body.

In vivo availability studies and initial toxicokinetic studies in CD-1 mice have revealed that RGT100-PEI can be detected in the plasma for up to 30 minutes after systemic administration. Major target organs for distribution are liver, spleen, and lung, where it can be detected for over 24 hours. Longer time spans have not yet been assessed.

Toxicokinetic data from 14-day dose range-finding studies, as well as 28-day toxicity studies, in CD-1 mice and cynomolgus monkeys have demonstrated that exposure to RGT100-PEI by intravenous (IV) and subcutaneous (SC) administration is dose-related but nonlinear, slight accumulation is seen over time, and elimination after IV administration is fairly rapid.

6.3.4 Toxicology

Dose range-finding studies (non-Good Laboratory Practice [GLP]) in mice (CD-1) and cynomolgus monkeys have been performed. In both species, 2-part studies have been performed, consisting of a dose range-finding portion with an escalating single dose administered by IV or SC injection, followed by a fixed, repeated dose portion with 3 dose level (DLs) administered for 14 days (dosed every other day). The studies included toxicokinetics, analyzing RGT100-PEI levels in plasma on the first and last days of treatment, and histopathology of selected organs and the injection sites.

In addition, non-GLP studies have been performed in mice to evaluate clinical effects (blood biomarkers: cytokines, hematology, and clinical chemistry) and desensitization after repeated dosing of RGT100-PEI.

Two 28-day IV repeated dose, GLP toxicity studies have also been performed in mice and cynomolgus monkeys. In both studies, the dosing frequency of RGT100-PEI was every other day. Both studies included a toxicokinetic part, analyzing RGT100-PEI plasma levels on the first and last days of treatment. Safety pharmacology assessments were also built into the studies. Overall, the studies demonstrated good tolerability up to the maximal feasible doses in both species, with no observed adverse effect levels (NOAELs) of 1.6 mg/kg and 0.16 mg/kg for mice and monkeys, respectively.

6.4 Dose Selection and Justification

Based on the pharmacodynamic data and the dose/tumor size correlation observed (refer to IB, Section 4.2.1.4), 0.2 mg RGT100 can be considered a pharmacologically active dose for intratumoral injection when extrapolated from tumors in mice to a human lesion with a diameter of 20 mm.

Accordingly, a starting dose (DL1) of 1 mL (0.2 mg) of RGT100-PEI is being considered for the first-in-human clinical study of intratumoral/intralesional injection into cutaneous or hepatic lesions. Results from the 4-week toxicity studies in mice and cynomolgus monkeys suggest the following NOAELs and human equivalent doses (HEDs) after IV administration of RGT100-PEI:

- NOAEL/maximum tolerated dose (MTD) in mice = 1.6 mg/kg IV → HED = 0.13 mg/kg
- NOAEL in cynomolgus monkeys = 0.16 mg/kg → HED = 0.05 mg/kg
- MTD in cynomolgus monkeys = 1.5 mg/kg → HED = 0.5 mg/kg

Leakage of RGT100-PEI from the lesion after intratumoral injection is expected to be marginal because, after intratumoral injection in mice, 100% of the compound remained local

(refer to IB, Section 4.2.1.4). However, for the calculation of safety margins, complete leakage (eg, accidental administration into a blood vessel) was used as a worst case assumption. A theoretical complete leakage from a cutaneous lesion was mimicked in the SC toxicity studies. Local toxicity after direct injection into the liver was not examined, but results from a preliminary biodistribution study showed that most RGT100 is found in the liver after IV injection. Therefore, the IV toxicity data can be used to extrapolate the safety of leakage into healthy liver tissue.

Thus, assuming a theoretical complete systemic ad hoc exposure after intratumoral administration, a starting dose of 1 mL (0.2 mg RGT100) in a 60 kg human (0.0033 mg/kg) would lead to the following safety factors (calculated with unrounded raw data):

- Safety factor for mouse NOAEL/MTD: 0.13/0.0033 = 40
- Safety factor for cynomolgus monkey NOAEL: 0.05/0.0033 = 15
- Safety factor for cynomolgus monkey MTD: 0.5/0.0033 = 150

Of note, the safety factor of 40 for the mouse has been calculated based on the NOAEL/MTD of 1.6 mg/kg (HED = 0.13 mg/kg) observed in the pivotal 4 week toxicity study. Nevertheless, in single-dose IV studies in mice (LPT studies 32867 and 33090) at a dose of 2 mg/kg (HED = 0.16 mg/kg) or 3 mg/kg RGT100-PEI, acute clinical signs and mortality were observed within 2 hours after administration of RGT100-PEI. Therefore, the safety factor for the minimum lethal dose is 48. Because this safety factor is based on the worst case assumption of complete leakage, it is considered acceptable for the intended patient population with advanced cancer.

6.5 Clinical Experience with RGT100-PEI

There are no prior or ongoing clinical trials in humans investigating RGT100-PEI. The first-in-human trial will evaluate the safety, tolerability, and anti-tumor activity of intratumoral/intralesional injections of RGT100-PEI in subjects with selected advanced or recurrent tumors.

6.6 Potential Risks and Benefits

Given that RGT100-PEI has not yet been tested in humans and this is a first-in-human study, the potential side effects of injecting this drug into humans are unknown. To date, RGT100-PEI has shown an acceptable safety profile for intratumoral/intralesional injection in animals. There has been a lack of any major side effects or toxicities in animal treated with RGT100-PEI.

Potential risks associated with the administration of RGT100-PEI based on nonclinical data may include the following:

- Immune-related adverse events (irAEs) (including colitis, hepatitis, thyroid complications) based on the fact that the mode of action of RGT100 revolves around the immune system
- Hypersensitivity reactions at the injected tumor site (skin rashes, pruritus or urticarial, flu-like symptoms such as fever, chills, muscle aches, fatigue, and headache, diarrhea, nausea and vomiting and low blood pressure)

- Altered liver function (increases in alanine transaminase [ALT], aspartate transaminase [AST], and lactate dehydrogenase [LDH], decreases in erythrocyte counts, hemoglobin levels, and hematocrit)
- Local reactions at the injected tumor site (swelling, induration, and erythema)

6.7 Rationale for the Study

Nonclinical studies have demonstrated that RGT100-PEI, a cytosolic receptor molecule for intratumoral/intralesional injection, induces a potent innate as well as adaptive immune response based on RIG-I activation in nonclinical studies.

This Phase 1/2 multicenter, first-in-human, open-label, dose escalation study will evaluate the safety, tolerability, and anti-tumor activity of intratumoral/intralesional injections of RGT100-PEI in subjects with selected advanced or recurrent tumors.

Results from this Phase I/II study will support further clinical development of RGT100-PEI. This study will be performed in compliance with the protocol, International Council for Harmonisation (ICH) Good Clinical Practice (GCP),[5] and local regulatory requirements. Aspects of the study concerned with manufacture and labelling of RGT100-PEI will meet the requirements of Good Manufacturing Practice.

7 STUDY OBJECTIVES AND ENDPOINTS

7.1 Objectives

The primary objectives of the study are to:

- Evaluate the safety and tolerability of intratumoral/intralesional injections of RGT100-PEI
- Determine the recommended Phase 2 dose (RP2D) feasible for intratumoral/intralesional injections of RGT100-PEI

The secondary objective of the study is to:

- Explore preliminary anti-tumor activity of RGT100-PEI on injected and un-injected tumor lesions

The exploratory objectives of the study are to:

- Investigate the type of cytokine release in plasma upon treatment
- Investigate the pharmacokinetics (PK) of RGT100-PEI in subjects with advanced or recurrent tumors
- Explore potential predictive biomarkers of tumor responses upon intratumoral/intralesional RGT100-PEI therapy*
- Evaluate the impact of RGT100-PEI therapy on immune infiltration of injected tumors*

*These exploratory objectives will be evaluated in a separate sub-study. Results of these exploratory investigations will not be presented as part of the main Clinical Study Report.

7.2 Endpoints

The primary endpoints of the study are:

- Incidence and severity of treatment-related adverse events (AEs) and laboratory abnormalities, graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03 criteria
- Occurrence of serious adverse events (SAEs)
- Occurrence of treatment discontinuation due to treatment-related AEs
- Incidence and severity of dose-limiting toxicities (DLTs)

The secondary endpoint of the study is:

- Objective response rate as evaluated radiologically using immune-related Response Evaluation Criteria in Solid Tumors (irRECIST)

The exploratory endpoints of the study are:

- Type of cytokine release in plasma
- Plasma concentrations of RGT100-PEI and, where feasible, standard PK parameters
- Presence of predictive biomarkers of tumor responses*
- Evidence of immune infiltration of injected tumors*

*These exploratory objectives will be evaluated in a separate sub-study. Results of these exploratory investigations will not be presented as part of the main Clinical Study Report.

8 INVESTIGATIONAL PLAN

8.1 Overall Study Design and Plan: Description

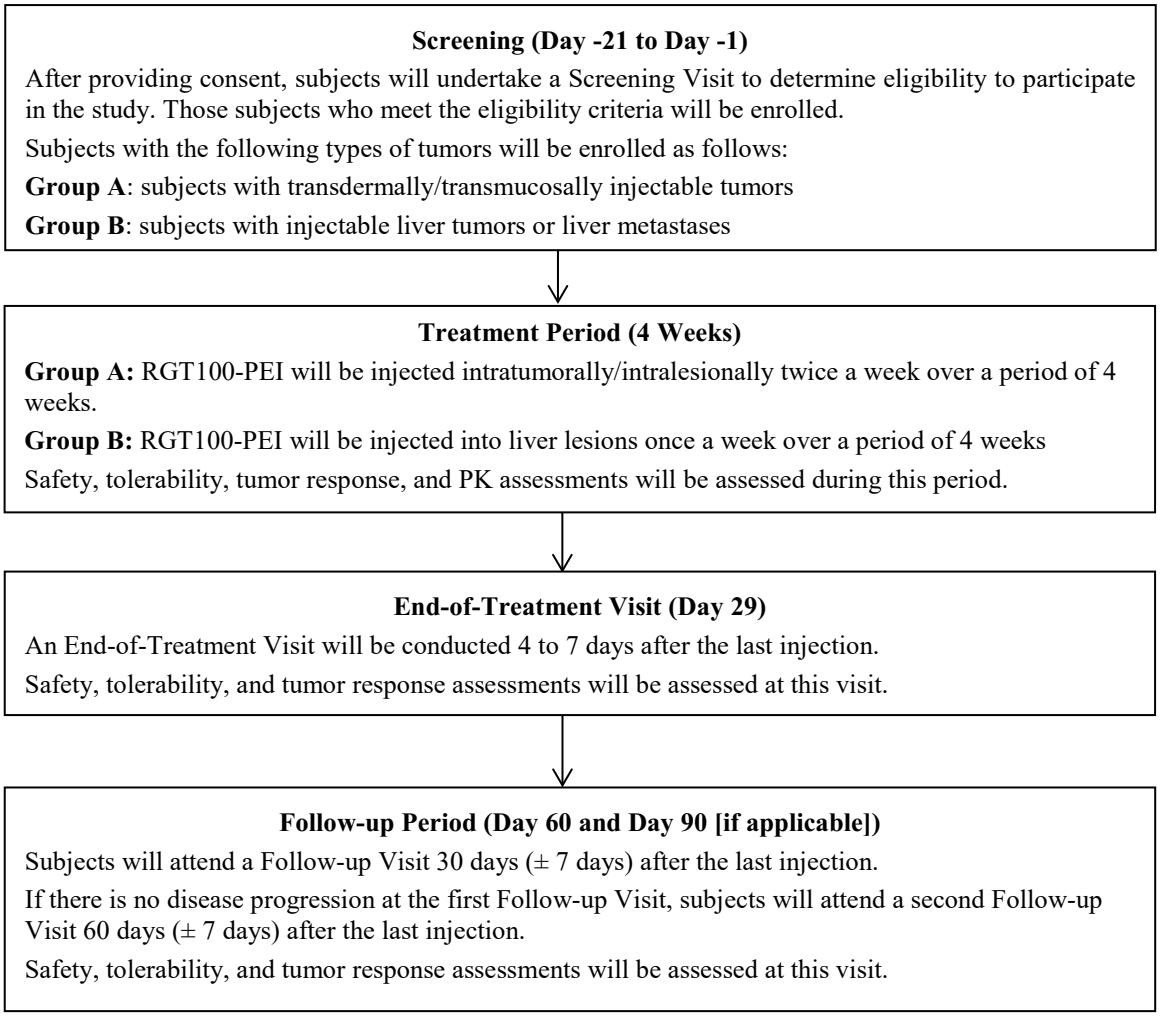
This is a Phase I/II multicenter, first-in-human open-label, dose escalation study to evaluate the safety, tolerability, and anti-tumor activity of intratumoral/intralesional injections of RGT100-PEI in subjects with selected advanced or recurrent tumors.

The study will be conducted in 2 groups (Group A and Group B) with each group consisting of a dose escalation phase followed by an expansion phase.

Each phase of the study will consist of a screening period, treatment period, end of treatment visit and a follow-up period.

A schematic diagram of the study design is presented in Figure 1.

Figure 1: Study Design



8.1.1 Dose Escalation Phase

This phase of the study will investigate increasing DLs of RGT100-PEI to determine the MTD or maximum feasible dose (MFD). The MFD is the highest dose volume that is safe and tolerable, and is feasible for intralesional/intratumoral injection. The MFD may be lower

than the MTD. The MTD (or MFD if lower than MTD) will define the RP2D to be used in the expansion phase of the study.

RGT100-PEI will be at a fixed concentration of 0.2 mg/mL and dose escalation will be based on the total volume of RGT100-PEI injected per subject. The planned DLs to be investigated in this phase of the study are:

- DL1: 0.2 mg = 1 mL
- DL2: 0.4 mg = 2 mL
- DL3: 0.6 mg = 3 mL
- DL4: 0.8 mg = 4 mL*

*Dose escalation may continue if deemed appropriate by the Safety Review Committee (SRC) (refer to Section 10.5 for SRC details). The corresponding next dose levels beyond the above and the supporting safety data to continue will be described within a substantial amendment to this protocol which will only be implemented once approval has been received.

It is not expected that a MTD will be reached at the proposed dose levels. It is more likely that a maximum feasible dose will be reached before the MTD, defined as the maximum volume which can be injected into a lesion. Experience with other compounds which are also injected intratumorally/intralesionally was taken into consideration when planning the proposed dose levels and the initially proposed highest dose. However according to the nature of a phase I trial the maximum tolerable and/or feasible dose level are yet unknown and cannot be predicted.

The specific properties of RGT100-PEI being an immune-modulating drug is taken into consideration and safety will be closely monitored to detect any early signs of toxicity in general and dose limiting toxicities in particular.

For each DL the second and subsequent subject treated will start treatment at least 7 days following the first subject's injection to allow initial assessment of safety data by the treating Investigator and to report any serious potential safety concerns such as DLTs.

Group A will commence dosing first. Group B will commence dosing once DL2 has been shown to be safe and well tolerated in Group A subjects. Dosing will then commence at DL2 for Group B.

A standard 3 + 3 design (Table 1) will be used for dose escalation (3 subjects per DL group, with the potential to add an additional 3 subjects to a DL group if toxicity is observed).

Table 1: Standard 3+3 Dose Escalation Scheme

Number of Subjects with a DLT at a Given Dose Level (during the DLT period)	**Action**
0 out of 3 subjects experience a DLT	Escalate to the next DL
1 out of 3 subjects experiences a DLT	Enter 3 additional evaluable subjects at the current DL for a total of 6 evaluable subjects
1 out of 6 subjects experiences a DLT	Escalate to the next DL

2 or more subjects (up to 6 subjects) experience a DLT	Dose escalation will be stopped (at this DL the MTD has been exceeded or declared)

Abbreviations: DL = dose level; DLT = dose-limiting toxicity; MTD = maximum tolerated dose.

If more than 2 subjects experience a DLT at a particular DL, a lower (or intermediate) DL may be explored to better define the MTD. Refer to Section 8.4.6 for DLT definition and Section 8.4.7 for MTD definition.

Approximately 9 to 24 subjects will be treated in each group during the dose escalation. All types of tumors that are accessible for intratumoral/intralesional injection may be treated in the dose escalation part of the study.

8.1.2 Expansion Phase

The expansion phase will require an amendment to this protocol after the escalation phase has been successfully completed to investigate the treatment with RGT100-PEI in additional 15 subjects in up to 4 different tumor indications (up to 60 subjects in total). The expansion phase will continue to be conducted according to the same Groups (Group A for cutaneous lesions and Group B for liver lesions) and according to the same treatment and examination schedule as the escalation phase.

Following the escalation phase all available safety and efficacy data as well as feasibility will be assessed and summarized in a safety report. This report will also include the selection and justification for the planned tumor indications as well as the RP2D for the expansion phase. The safety report together with an amended protocol will be submitted to authorities and the expansion phase will only start if the proposed RP2D and tumor indications are accepted by authorities.

8.1.3 Extended Treatment Period

Subjects with partial response (PR) or stable disease (SD) at 8 weeks and no clinical deterioration will be allowed to be treated with a second cycle of RGT100-PEI for an additional 4 weeks. This will be based on Investigator decision and subject agreement, both documented in the subject file and electronic case report form (eCRF).

These subjects must have completed initial participation with acceptable toxicity. During further therapy, subjects will continue to be assessed for safety and tolerance, as well as for preliminary evidence of anti-tumor efficacy of RGT100-PEI. For details of study assessments to be performed for the extended treatment period, refer to Appendix 17.1 (Group A) and Appendix 17.2 (Group B).

8.2 Discussion of Study Design

The study is designed as a Phase I/II, open-label, repeat dose escalation study of RGT100-PEI in subjects with advanced or recurrent tumors. The data will be used to assess safety, tolerance, and tumor response of repeat doses of RGT100-PEI.

The study design exposes a small number of subjects to each dose of RGT100-PEI and uses different subjects for each cohort. A low starting dose is used to evaluate safety with minimal

risk to subjects. Initially a single subject will be dosed, 1 week before dosing the remaining subjects in the cohort.

A SRC will meet to review the safety results from a given cohort, before escalating to the next dose in a new cohort. Refer to Section 10.5 for SRC details.

A conventional 3 + 3 design is considered a safe design for a dose escalation study in this subject population.

Frequent assessments of safety will be conducted to support the primary objective for conducting the study. The Investigator will review safety and tolerability data to ensure that subjects will only receive another dose of RGT100-PEI in the subsequent treatment period if the dose is being tolerated. Dose escalation meetings will be held to review safety and tolerability data for all subjects before dose escalation and before entering the expansion phase.

Tumor response also will be assessed to support the secondary and exploratory objectives of the study.

No placebo arm is offered to this subject population for ethical reasons.

8.3 Selection of Study Population

8.3.1 Number of Planned Subjects

The number of subjects enrolled in the dose escalation phase will depend on the number of DLTs observed in each cohort and the number of DLs evaluated. Approximately 9 to 24 subjects are planned to be treated each in Group A (cutaneous lesions) and Group B (liver lesions) during dose escalation.

In the expansion phase, an additional 60 subjects (4 groups of 15 subjects each) in each tumor-specific group (60 subjects in total) will be treated at the RP2D for further tolerability and preliminary efficacy assessments. No formal power calculations were performed to obtain the suggested sample size. During further therapy, subjects will continue to be assessed for safety and tolerance, as well as for preliminary evidence of anti-tumor efficacy of RGT100-PEI. Hence the number of subjects has been based on the desire to obtain adequate safety, tolerability, anti-tumor activity, and PK data while exposing as few subjects as possible to the investigational product and procedures.

The tumor type to be included in each cohort will be determined following the completion of the dose escalation phase and will be based on the preliminary anti-tumor activity results from dose escalation. The expansion phase is not intended to provide definitive results but will give a better indication of safety in the chosen subject population and will provide preliminary efficacy data.

If a subject does not meet the list of key inclusion/exclusion criteria the first time, re-screening is allowed, as long as dose escalation or expansion phase is recruiting.

8.3.2 Inclusion Criteria

A subject will be eligible for inclusion in this study if all of the following criteria apply:

1. Male or female aged ≥ 18 years

2. Subjects with histologically or cytologically confirmed diagnosis of advanced or recurrent tumors (including lymphomas) for whom all standard treatments have been used or are not feasible or RGT100-PEI is a suitable treatment option and:
    a. For Group A: has cutaneous, SC, or lymph node injectable tumors
    b. For Group B: has injectable liver tumors or liver metastases
3. Eastern Cooperative Oncology Group (ECOG) performance status 0-2
4. Life expectancy > 3 months as assessed by the Investigator
5. Adequate organ function:
    a. Bone marrow function: Hemoglobin ≥ 10 g/dL (pre-treatment transfusion allowed); lymphocyte count ≥ 0.5×10^9/L; absolute neutrophil count ≥ 0.5×10^9/L; platelet count ≥ 75×10^9/L
    b. Hepatic function: AST and ALT ≤ 2 × upper limit of normal (ULN) (3 × ULN in the case of liver metastases); bilirubin ≤ 1.5 × ULN (2 × ULN in case of liver metastases)
    c. Renal function: creatinine < 1.5 × ULN and/or creatinine clearance ≥ 50 mL/min (Cockroft and Gault)
6. Negative serum pregnancy test within 2 weeks before first dose of study drug if the subject is a woman of childbearing potential. Subjects and subjects' partners of childbearing potential must agree to use birth control consistently and correctly during the study and for at least 6 months after the last study drug application.
7. At least 1 measurable lesion per RECIST 1.1 and 1 separate injectable lesion with diameter ≥ 1 cm but < 7 cm
8. Ability to provide written informed consent before any study drug-related screening procedures being performed

8.3.3 Exclusion Criteria

A subject will not be eligible for the trial if any of the following apply:

1. Any tumor-directed therapy within 4 weeks before study treatment
2. Treatment with investigational drugs within 4 weeks before study enrolment
3. Systemic steroids at a dose of > 10 mg of prednisolone, > 2 mg of dexamethasone a day or equivalent, except topical (inhaled, topical, nasal) for the last 28 days and ongoing
4. Subjects with rapidly progressing disease (as determined by the Investigator)
5. Ongoing immune-related adverse events (irAEs) and/or AEs ≥ grade 2 not resolved from previous therapies except vitiligo, stable neuropathy grade 2, hair loss, and stable endocrinopathies with substitutive hormone therapy
6. Within 4 weeks of major surgery
7. Prior splenectomy
8. Documented history of active autoimmune disorders requiring systemic immunosuppressive therapy

9. Primary or secondary immune deficiency
10. Active allergy requiring systemic medication or active infections requiring anti-infectious therapy
11. Seropositive (except after vaccination) for human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV)
12. Clinically significant cardiac disease including heart failure (New York Heart Association, Class III or IV), pre-existing arrhythmia, uncontrolled angina pectoris, or myocardial infarction within 1 year before study entry
13. Dementia or altered mental status that would prohibit informed consent
14. Other severe, acute, or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study assessed by the Investigator
15. History of stroke, seizures, encephalitis, or multiple sclerosis
16. Gastric ulcer or inflammatory bowel disease or Crohn's disease or ulcerative colitis in the last 6 months
17. Active drug or alcohol abuse
18. Pregnant or breast feeding

8.3.4 Removal of Subjects From Therapy or Assessments

8.3.4.1 Subject Withdrawal

Subjects may stop study drug for any of the following reasons:

- Subject request
- Use of non-permitted concurrent therapy
- Non-compliance with the study drug or study schedule
- Lost to follow-up
- Occurrence of AEs not compatible with the continuation of the study, in the Investigator's opinion, or unacceptable to the subject to continue
- Investigator request
- Intercurrent illness
- Sponsor request
- Treatment failure

Subjects are free to withdraw from the study at any time without providing reason(s) for withdrawal and without prejudice to further treatment. The reason(s) for withdrawal will be documented in the eCRF.

Subjects withdrawing from the study will be encouraged to complete the same final evaluations as subjects completing the study according to this protocol, particularly safety

evaluations. The aim is to record data in the same way as for subjects who completed the study.

Reasonable efforts will be made to contact subjects who are lost to follow-up. These efforts must be documented in the subject's file. The reason for early withdrawal from treatment should be clearly documented in the medical records. An end of treatment visit assessment should be performed and the results of the evaluations and observations reported in the eCRF.

Consent withdrawal means that a subject has expressed a wish to withdraw from the study. Under these circumstances, the site needs to document all relevant discussions in the subject notes and notify the contract research organization (CRO), which will allow the CRO to mark all future eCRFs as not applicable. Under these conditions, Investigators are still responsible to follow up any SAEs until resolution.

The Sponsor has the right to terminate the study at any time in case of SAEs or if special circumstances concerning the study drug RGT100-PEI or the company itself occurs, making further treatment of subjects impossible. In this event, the Investigator(s) will be informed of the reason for study termination.

8.3.4.2 Subject Replacement

Subjects who do not comply with the protocol or who withdraw consent may be replaced.

In the dose escalation phase, a subject will not be replaced if they come off treatment due to a DLT. They are, however, not evaluable (NE) for the primary endpoint if they withdraw for other reasons than safety/toxicity before completing 4 weeks of treatment (the DLT period), and will need to be replaced. Withdrawn subjects will not receive further treatment with RGT100-PEI, but will be followed up unless they withdraw consent.

If subjects in Group A miss more than 2 injections or subjects in Group B miss more than 1 injection due to drug-related toxicity (but not qualifying for a DLT), these subjects will be replaced in the corresponding group for the purpose of DLT evaluation.

In the expansion phase, subjects are evaluable if they have received at least 1 injection of RGT100-PEI. Non-evaluable subjects for the primary endpoint of assessment of toxicity may be replaced.

8.3.4.3 Pregnancy

Pregnancies (in a participant or partner) occurring while participating in this trial require expedited reporting. A pregnancy form should be completed and faxed to INC Research Safety & Pharmacovigilance within 24 hours of the Investigator becoming aware of the pregnancy. All reported pregnancies should be followed and the outcome reported using the same form. If an AE occurs in relation to the pregnancy, it has to be noted on the pregnancy form and recorded within the eCRF. If an SAE occurs in relation to the pregnancy, it has to be noted on the pregnancy form, recorded within the eCRF, and immediately reported to INC Research Safety & Pharmacovigilance as described in Section 10.4.4.3.

The Investigator must make all reasonable efforts to follow the pregnancy until its end and will report all outcomes associated with the pregnancy to the Sponsor. If the outcome of the pregnancy meets any of the criteria for seriousness, it must also be reported as an SAE. Examples of pregnancy outcomes that are SAEs include reports of:

- Congenital anomalies or developmental delay, in the fetus or the child

- Fetal death and spontaneous abortion
- Suspected adverse reactions in the neonate that are classified as serious

In the situation of pregnancy of the female partner of a male study subject, consent for the release of medical data should be obtained from the female partner to allow collection of information on the outcome of the pregnancy.

Women who become pregnant should be withdrawn from trial treatment immediately.

8.3.5 Protocol Deviations and Waivers to Entry Criteria

Protocol adherence is a fundamental part of the conduct of a clinical study. Changes to the approved protocol need prior approval unless for urgent safety reasons.

Systems for prospectively approving protocol deviations, in order to effectively widen the scope of a protocol must not be used. Doing so might erode the scientific and ethical value of the protocol and its authorization and might have an impact on the processes put in place for the care and safety of the study subjects.

Investigators must contact the CRO to obtain guidance and/or clarification as necessary if unsure whether the subject satisfies all the entry criteria and to clarify matters of clinical discretion. The CRO will contact the Sponsor or Lead Investigator as necessary.

Protocol waiver to enter a subject who does not satisfy the selection criteria are not allowed. The Investigator must document and explain any deviations/violations from the approved protocol. The Investigator should promptly report any important violations that might impact subject safety, data integrity, or be a possible serious breach (refer to Section 11.3) to the Sponsor/CRO.

8.4 Investigational Products

8.4.1 Investigational Products Administered

RGT100-PEI is to be administered by intratumoral/intralesional injection into cutaneous, subcutaneous, nodal and or intrahepatic lesions that are visible, palpable or detectable by imaging guidance (eg, ultrasound).

In Group A: RGT100-PEI will be administered into cutaneous, subcutaneous or nodal lesions by trained site staff.

In Group B: RGT100-PEI will be administered into liver lesions under image guidance (eg, ultrasound) by a radiologist or another experienced physician.

There will be no interruption between treatments, except as described in the dose modification and dose delay section (Section 8.4.8.4).

8.4.1.1 Injection of RGT100-PEI – Tumor Selection

Injections should be targeted at viable tumor tissue and not at necrotic tissue. The Investigator may use his or her judgement to determine which tumors are accessible and injectable, and select an index lesion for injection. The accessibility and injectability will be assessed in accordance with routine medical practice (e.g., by physical palpation [SC lesions], by ultrasound or computerized tomography [CT] guidance if necessary [liver lesions]; in Germany only ultrasound guidance may be used). There will be documentation of the method

of assessment and various characteristics of the selected lesion (presence or absence of necrosis, ulceration, progression and prior local therapies).

It is up to the Investigator's discretion to select the index lesion and the lesion that serves as reference lesion.

There should be at least 1 measurable lesion left untreated, to meet the secondary endpoint of assessing changes in untreated disease and for irRECIST-assessment.

There is no limit to the proximity of the lesions selected for injection, except:

- Injected lesion(s) must be clearly distinguishable
- The un-injected lesion selected for observation (reference lesion) must be sufficiently remote from an injected lesion to allow for evaluation over a 60/90 day duration of the study.

The Investigator should make all efforts to apply the whole volume of drug into the chosen index lesion especially during the escalation phase.

The total volume of drug should be injected into the chosen index lesion. All lesions will be injected either directly or under image guided control (e.g., ultrasound). At each injection, the operator should collect the following information:

- Location of the lesion in the body
- Size of the injected lesion
- NCI CTCAE grading inflammation at the site and time of injection, if any, before and after injection
- Type of lesion injected (skin, lymph node, liver)
- Planned volume to be injected in the lesion (for eventual intent-to-treat analysis)
- Actual volume injected in the lesion (for eventual per dose received analysis)

The total volume of the corresponding dose level should be injected into the chosen index lesion. Only in cases where this is not possible the investigator will be allowed to split the total volume of a DL and inject the remaining volume into another lesion. The total volume of the applicable DL must not be exceeded.

In the instance that a lesion exhibits complete response (CR) during the 4 weeks of therapy, the Investigator will be allowed to choose additional lesions for injection. The Investigator always needs to document which lesion(s) has/have been injected.

In order to avoid a non-injected target lesion becoming an injected lesion (and therefore rendering a subject as non-interpretable), it will be required that during the screening period the radiologist and the interventional radiologist (or Investigator) define together the non-injected lesions to be followed by irRECIST and list by priority order the lesion to be injected during the treatment.

Depending on the specific dose level and thus the amount of volume to be injected, the investigator should choose the index lesion for injection accordingly based on its size. The following algorithm below should be used as a guidance. However, it is up to the investigator to select an appropriate index lesion considered feasible for the volume to be injected, which may also be a bigger or smaller lesion.

- Dose level 1: 1.0 mL for lesions of 1.0 to 2.0 cm longest diameter
- Dose level 2: 2.0 mL for lesions of > 2.0 to 3.0 cm longest diameter
- Dose level 3: 3.0 mL for lesions of >3.0 to 5.0 cm longest diameter
- Dose level 4: 4.0 mL or more for lesions > 5 to 7 cm longest diameter

The measurement will be of the longest diameter of vital tumor tissue. In the event that it is not possible to assess the discrete size of the vital tissue, the maximum volume injectable should be pro-rated according to the percentage composition of necrotic tissue. The Investigator should also consider the tumor type and the tumor density in determining the lesion to be injected at the corresponding dose level.

8.4.1.2 Injection of RGT100-PEI – Preparation

RGT100-PEI dispersion is available in 10-mL ready-to-use vials with an extractable volume of 5 mL containing 0.2 mg/mL. The vials should be stored frozen at -20ºC (±5ºC).

The following steps should be taken before injection:

- Thaw RGT100-PEI vial(s) to room temperature. It is recommended to administer RGT100-PEI within 4 hours.
- Draw the desired amount of RGT100-PEI from the vial into a syringe directly before the injection. Note: the vials of RGT100-PEI are for single use only and any unused dispersion has to be discarded.
- Clean the lesion and/or surrounding areas with an alcohol swab or other appropriate agent and let dry.
- For intrahepatic lesions follow routine procedure for preparation of the injection site.
- Cutaneous and subcutaneous lesions: Anesthetic agents should be avoided if possible as there is no knowledge if there may be an interaction between RGT100-PEI and an anesthetic agent. In case of severe pain, the injection site may be treated with a topical anesthetic agent.

8.4.1.3 Procedures for first injection

It is up to the Investigator to decide the best mode of accessing the tumor to inject the drug. Some examples are:

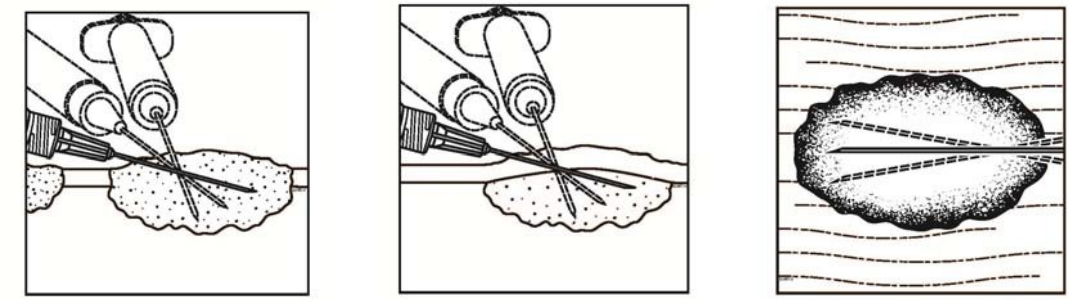
- Direct manual/visual control
- Ultrasound guidance
- CT guidance (not to be used in Germany)

Injection into liver lesions will be performed under image guidance. The total volume for injection will depend on the current DL in the dose escalation phase and on the RP2D during the expansion phase. In case several tumor lesions need to be injected, the minimum volume per injection and lesion should be 0.5 mL.

The site may utilize its routinely used needles and materials for both the cutaneous and liver lesion injections. For example: For cutaneous lesions a short needle for SC injections and for

liver lesions a 21-gauge spinal needle may be feasible for use. For liver lesion injections, local anesthesia should be considered according to the site's routine procedures together with ultrasound guidance.

Cutaneous lesions | Subcutaneous lesions | Nodal/liver lesions



The following steps should be taken for the injection:

- Determine and record the injection volume for the specific lesion.
- Using a single insertion point, inject RGT100-PEI along multiple tracks as far as the radial reach of the needle allows within the lesion to achieve even and complete dispersion. Multiple insertion points may be used if a lesion is larger than the radial reach of the needle.
- Disperse RGT100-PEI evenly and completely within the lesion by pulling the needle back without exiting the lesion. Redirect the needle as many times as necessary while injecting the remainder of the dose of RGT100-PEI. Continue until the full dose is evenly and completely dispersed.
- Use local policies to account for dead space and for flushing
- When removing the needle, withdraw it from the lesion slowly to avoid leakage or splash back of RGT100-PEI at the insertion point.
- Repeat these steps for other lesions that need to be injected. Use a new needle anytime the needle is completely removed from a lesion and each time a different lesion is injected.
- Cutaneous lesions only: apply pressure to the injection site with a sterile gauze according to routine procedures
- Intrahepatic lesions only: apply pressure to the injection site with a sterile gauze according to routine procedure.
- Dispose of all materials that have come in contact with RGT100-PEI (e.g., vial, syringe, needle, any cotton or gauze) in accordance with local institutional procedures.

The Investigator should note and record any unusual backpressure to the injection, any suspicion of leakage of injected material beyond the index lesion and any reasons why the predicted volume to be injected into an individual lesion was not achievable.

8.4.1.4 Procedures for further injections

The chosen index lesion will be injected at all scheduled visits. Only in cases where this is not possible the following lesion selection procedure will be followed:

The priority for tumor/lesion injection will be as follows:

- Previously un-injected lesions will only be selected for injection if the planned total volume for injection cannot fully be administered in the previously treated lesion (e.g., reduced lesion size). Previously un-injected tumors should be selected according to the following priority:
    1. Pre-existing lesions from the previous treatment visit that were not previously injected
    2. New lesions, newly measurable lesions and newly documented lesions which appear during the course of the study.

8.4.2 Identity of Investigational Product

The investigational medical product in this trial is RGT100-PEI, which is a double stranded 3-phosphate-RNA that binds to RIG-I (RGT100); *in vivo* JetPEI™ is the delivery vehicle. RGT100-PEI will be applied intratumorally/intralesionally. RGT100-PEI will be provided as ready-to-use formulation in 10-mL vials.

8.4.3 Packaging and Labelling

The manufacture and labelling of RGT100-PEI meets the requirements of Good Manufacturing Practice and will be performed at the manufacturing site and at the drug supplier.

8.4.4 Method of Assigning Subjects to Treatment Groups

Once written informed consent has been obtained, the subject will be issued with a unique screening number, which will be recorded in a Screening Log. Following screening, subjects who meet all the inclusion criteria and none of the exclusion criteria will be enrolled in to 1 of the groups of the study, and sequentially assigned to a dose cohort of RGT100-PEI during the dose escalation phase or to the RP2D for the expansion phase.

If appropriate, and with prior approval from the Sponsor or their designee, subjects who fail screening, or who pass screening but have not been treated, may be re-screened for subsequent cohorts.

8.4.5 Subject Enrolment and Slot Allocation

Slot allocation will be based on competitive enrolment. However, if there are any pre-allocated slots, the INCR Project Lead (PL) will notify the site personnel of the pre-allocated slots prior to each cohort review/opening. Another review of slots allocation will be done during Safety Review calls and Dose Escalation Meetings.

The site will notify the INCR PL via email as soon as a subject has been identified. The PL maintains a tracking overview of all communicated potential study subjects and will confirm the current status back to the site.

Sites are expected to identify potential subjects prior to the Dose Escalation Meetings and to be able to start consenting/screening procedures directly following the slot confirmation.

The email notification must include the following information:

- Subject initials and/or local tracking number (depending on site/local regulations)

- Tumor type
- Year of birth
- Planned Consent / Screening date and
- Anticipated start date

A slot will not be held for any subject if the above information is not available.

Ultimately, in case of dispute or unclarity, the INCR Project Lead (PL), in discussion and agreement with Rigontec, is in charge of slot allocation and has final say.

If a site does not confirm that their identified and slot allocated subject has consented within 5 business days of the cohort opening, the unclaimed slot will become competitive (unless otherwise agreed in writing between the site and INCR PL). The INCR PL will notify all open sites of the availability of competitive slots and all sites will be notified the same day when a competitive slot is claimed. This process will continue until the cohort is filled.

This information will be reiterated at the safety meetings or calls.

Once a subject has successfully completed screening, the Enrolment form must be completed and sent as an attachment to PPD. The INC Medical Monitor will check, evaluate the enrolment form and approve or reject to assure that only eligible subjects will be enrolled into the trial and into the correct cohort. The subject number, treatment and dose will be confirmed within 12 (business) hours per email.

8.4.6 Selection of Doses in the Study

The concentration of the RGT100-PEI particles in the injection dispersion is fixed to 0.2 mg/mL to achieve stable complexes. Volumes of RGT100-PEI will be escalated to provide total doses of, for example, 0.2, 0.4, 0.6, and 0.8 mg per injection.

The planned starting dose of 1 mL (0.2 mg) for the dose escalation phase of the study is based on pre-clinical data (refer to Section 6.4 for dose justification). To date there are no human data for RGT100-PEI.

Subsequent dose escalation will be based on the safety findings of the previous dose following review by the SRC (refer to Section 8.1.1 for dose escalation details).

8.4.7 Dose-limiting Toxicity

The DLTs and MTD are defined using the NCI CTCAE version 4.03.

Dose-limiting toxicities will be drug-related toxicities (considered related or possibly related to) and graded using NCI CTCAE version 4.03 occurring during the DLT period and include:

- Non-hematologic toxicity ≥ grade 3 (except diarrhea, nausea, and vomiting unless lasting >3 days despite optimal supportive care)
- Confirmed (with a second measurement after 24 hours) non-hematologic appropriately graded laboratory findings of grade ≥3 that were ≤ grade 1 at baseline
- Hematologic toxicity:

- Grade 4 neutropenia ≥ 5 days, or grade 3 neutropenia with fever (fever is > 38.4ºC)
- Grade 4 thrombocytopenia, or grade 3 thrombocytopenia lasting > 7 days or with bleeding

- Any other toxicity assessed as related to RGT100-PEI, and which, in the opinion of the study Investigator(s) and the Sponsor physician constitutes a DLT

The DLT period will be 4 weeks. Evaluation of the available safety data over this DLT period will be required from at least 3 subjects per DL and per Group (A or B) before a decision is made whether to enroll additional subjects at the same, or the next DL.

Note: Subjects withdrawn from the study before the completion of the DLT period for reasons other than DLT will be replaced.

During dose escalation, escalation to the next DL will occur only if the safety and tolerability (including DLTs) data are acceptable as determined during the dose escalation meetings by the SRC. If any change is made to the grade or causality of an AE during the study that may alter its DLT status, the Sponsor and SRC must be informed immediately as this may affect dose escalation decisions.

The following toxicities will not be considered as DLTs:

- Grade 3 AE of tumor flare (defined as local pain, irritation, or rash localized at sites of known or suspected tumor)
- Grade 3 irAE that resolves to grade ≤ 1 within 7 days.

8.4.8 Maximum Tolerated Dose, Maximum Feasible Dose, and RP2D

The MTD is defined as 1 DL below the DL where ≥ 2 of 6 subjects in a group experience a DLT for RGT100-PEI during the DLT period of 4 weeks. Maximum tolerated dose will be determined following discussion of all the relevant toxicity data by the SRC during the dose escalation meetings after each dose escalation step (refer to Section 10.5 for SRC details).

If ≤ 1 of 6 subjects in all dose levels experience a DLT, then an MTD will not have been reached in this group. In this case, the RP2D will be selected based on integrated evaluation of safety, tolerability, clinical benefit, and pharmacodynamic data, for all DLs tested.

Once the MTD has been defined, dosing at a higher dose must not occur in this group. Once the MTD has been exceeded, the cohort at the next lowest DL will be expanded to 6 subjects if necessary and only 3 subjects were involved before. The SRC are free to decide on an intermediate DL.

The MFD is the highest dose volume that is safe and tolerable, and is feasible for intralesional/intratumoral injection in the respective group. The MFD may be lower than the MTD. The MTD (or MFD if lower than MTD) will define the RP2D to be used in the expansion phase of the study.

8.4.9 Selection and Timing of Dose for Each Subject

8.4.9.1 Dose Escalation Phase

The planned DLs to be investigated are presented in Table 2 below.

Table 2: Dose Levels for RGT100-PEI Dose Escalation

Dose Level	RGT100-PEI Dose	Volume of RGT100-PEI to be Injected
1	0.2 mg	1 mL
2	0.4 mg	2 mL
3	0.6 mg	3 mL
4	0.8 mg	4 mL

RGT100-PEI will be administered by intratumoral/intralesional injection. A dose escalation meeting with the SRC (refer to Section 10.5 for SRC details) will be held to evaluate a dose escalation after all subjects in a DL per group have completed the DLT period.

Group A: Dosing for Group A (cutaneous lesions) will start first. The total dose will be administered each on 2 separate days of every week (Mondays and Thursdays or Tuesdays and Fridays) for a maximum of 4 weeks.

Group B: Dosing for Group B (liver lesions) will start once DL2 has proven to be safe in Group A. Dosing in Group B will then start at DL2.The total dose will be administered on 1 day of every week for a maximum of 4 weeks.

8.4.9.2 Expansion Phase

RGT100-PEI will be administered by intratumoral/intralesional injection at the RP2D established in the dose escalation phase.

The timing of dose administration will be as per the dose escalation phase.

8.4.9.3 Extended Treatment Phase

Subjects with PR or SD at 8 weeks and no clinical deterioration will be allowed to be treated with a second cycle of RGT100-PEI for an additional 4 weeks. This will be based on Investigator decision and subject agreement.

The selected dose and timing will be as per the recently completed previous treatment period in which subjects were enrolled. For details of study assessments to be performed for the extended treatment period, refer to Appendix 17.1 (Group A) and Appendix 17.2 (Group B).

8.4.9.4 Dose Modification and Dose Delay

Every effort should be made to administer trial treatment on the planned schedule. If a dose is missed, the dose should be administered as soon as possible. There should be at least 2 days between consecutive injections.

Treatments visits can be moved by 1 day when necessary for logistical purposes.

The End-of-Treatment Visit (Day 29) can be moved by ± 3 days and follow-up visits can be moved by ± 7 days when necessary.

The toxicity of treatment must be recorded before the administration of the next dose and graded according to the current version of the NCI CTCAE version 4.03. If individual

subjects experience treatment-related toxicity, subsequent trial treatment may be delayed as described below. Dose reductions are not permitted.

For grade 3, grade 4, or intolerable grade 2 toxicities, the treatment will be interrupted until recovery to ≤ grade 1 or to baseline. Trial treatment will be permanently discontinued if the subject fails to recover to this extent within 3 weeks. Dose reductions or modifications are not allowed in both, the dose escalation and expansion phase.

If a subject cannot tolerate treatment despite the dose delays, trial treatment will be discontinued. The subjects will then undergo end of treatment visit and will be followed up according to plan.

In the event of a RGT100-PEI overdose (defined as any dose above the applicable DL), the Investigator should report such an overdose via the SAE form immediately and closely monitor the subject for AEs. The Investigator will use clinical judgement to treat any overdose.

Treatment is to be discontinued prematurely in the following situations:

- Complete response
- All injectable lesions have disappeared
- Intolerance of study treatment
- Subject withdrawal

8.4.10 Blinding

Blinding will not be used in this study. An open-label design is appropriate to study the effects of RGT100-PEI at various doses in this Phase I/II study, and allow unblinded review of study data to make informed decisions regarding dose escalation.

8.4.11 Prior and Concomitant Therapy

Concomitant medication may be given as medically indicated. All subjects will be asked to provide a complete list of concomitant medication including prescription, over-the-counter, complementary, and alternative medications that have been taken within 4 weeks before the first treatment visit. They must also inform the Investigator about any new medication started while on the trial.

Details (including indication, doses, frequency, and start/stop dates) of concomitant medication taken during the trial, until the completion of the end of treatment visit, must be documented in the medical record and the appropriate eCRF.

8.4.12 Prohibited Medication/Therapy

Subjects should not be prescribed any other anti-cancer or investigational therapies while participating in this study. The use of immunosuppressive therapy and systemic corticosteroids is strongly discouraged on the trial. Subjects are allowed to use a dose of ≤ 10 mg of prednisolone, ≤ 2 mg of dexamethasone or equivalent other steroid. If an indication for this treatment arises during study treatment this should first be discussed with the Sponsor or Principal Investigator for alternatives. In case of no alternatives the trial treatment is to be permanently discontinued. However, in case a subject is known to have

allergy against contrast media, premedication with steroids to prevent potential hypersensitivity are allowed.

8.4.13 Treatment Compliance

RGT100-PEI will be administered by the Investigator (or designee) and administration information will be recorded in the appropriate eCRF. Only study site staff delegated by the Investigator to dispense/administer RGT100-PEI may dispense and/or administer the investigational product. The date and time of dosing will be captured in the eCRF.

8.4.14 Safety measures and observation of subjects

After each injection of RGT100-PEI subjects will be closely monitored for safety as included in the schedule of assessment (see section 9 below). For the first injection subjects must be monitored for at least 6 hours after injection including assessment of vital signs immediately before injection and 15, 30, 45, 60, and 90 minutes and 2, 3, 4, and 6 hours after injection and must stay overnight for observation in the hospital. For subsequent injections subjects must be monitored for at least 4 hours including assessment of vital signs before and hourly after injection.

Investigators may also consider an overnight stay for the following injections if deemed appropriate.

Facilities and equipment for resuscitation must be readily available at the investigational sites when performing injections with RGT100-PEI and during the observation time following each injection to shorten reaction times in case of severe hypersensitivity. Investigators performing or supervising injections should be trained accordingly in such measures and use of equipment.

9 TIMING OF STUDY PROCEDURES

Participants will undergo a screening period of up to 3 weeks. The study treatment period will be approximately 4 weeks (shorter if the subject withdraws, and longer if there are delays to dosing due to toxicities). All subjects will be followed up for 30 days following their final dose of RGT100-PEI or until resolution or stabilization of all treatment-related AEs, whichever is later. If no progressive disease (PD) is seen after a 30-day follow-up period, subjects will be in follow-up for another 30 days (total of 60 days after last injection), which defines the end of the trial. Further survival follow-up will be performed every 3 months for a period of 24 months outside of the main study.

Subjects will provide written informed consent before any study-related procedures are performed. The timing of study assessments is provided in Table 3 for Group A and Table 4 for Group B.

Table 3: Group A (Cutaneous) Schedule of Assessments

Trial Visits, Investigations, and Interventions	**Screening**	**Treatment Period**								**End-of-Treatment Visit**	**Follow-up Visit**	
	Day -21 to Day -1	**Week 1**		**Week 2**		**Week 3**		**Week 4**		**Week 5**	**Week 9**	**Week 13[q]**
		Day 1 **Inj 1**	Day 4 **Inj 2**	Day 8 **Inj 3**	Day 11 **Inj 4**	Day 15 **Inj 5**	Day 18 **Inj 6**	Day 22 **Inj 7**	Day 25 **Inj 8**	Day 29 (±3 days)	30 days after last injection (±7 days)	60 days after last injection (±7 days)
RGT100-PEI injection [a]		+	+	+	+	+	+	+	+			
Informed Consent	+											
Medical History	+											
Eligibility Criteria	+											
Pregnancy Test [b]	+									+		
ECG	+									+	+	+
Physical Examination [c]	+	+		+		+		+		+	+	+
ECOG Performance Status	+	+		+		+		+		+	+	+
Vital Signs [d]	+	10+	10+	5+	5+	5+	5+	5+	5+	+	+	+
Overnight observation		+	As deemed appropriate by the investigator									
Height/Weight	+											
Hematology [e]	+	+ +	+	+ +	+	+ +		+ +		+	+	+
Clinical Chemistry [e]	+	+ +	+	+ +	+	+ +		+ +		+	+	+
Urinalysis [e]	+	+ +	+	+ +	+	+ +		+ +		+	+	+
Autoimmunity [f]	+					+				+	+	
Infectious Diseases [g]	+									+	+	
Immunoglobulins [h]	+					+				+	+	
Immuno-monitoring [i]	+	+ + +							+ + +	+	+	+
PK (RGT100-PEI) [j]		**7+**							**7+**			
Mutation Status [k]	+											
Tumor Assessment and Photography [l]	+	+		+		+		+		+	+	+

Trial Visits, Investigations, and Interventions	**Screening**	**Treatment Period**								**End-of-Treatment Visit**	**Follow-up Visit**	
	Day -21 to Day -1	**Week 1**		**Week 2**		**Week 3**		**Week 4**		**Week 5**	**Week 9**	**Week 13[q]**
		Day 1 **Inj 1**	Day 4 **Inj 2**	Day 8 **Inj 3**	Day 11 **Inj 4**	Day 15 **Inj 5**	Day 18 **Inj 6**	Day 22 **Inj 7**	Day 25 **Inj 8**	Day 29 (±3 days)	30 days after last injection (±7 days)	60 days after last injection (±7 days)
Imaging [m]	+										+	+
Biopsy [n]		+								+		
Adverse Events [o]	+	+	+	+	+	+	+	+	+	+	+	+
Concomitant Medication [p]	+	+	+	+	+	+	+	+	+	+	+	+

Abbreviations: ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; Ig = immunoglobulin; Inj = injection; PK = pharmacokinetics; PR = partial response; SD = stable disease.

a. Subjects with PR or SD at week 9 (30 days after last injection) will be allowed to be treated with a second cycle of RGT100-PEI for an additional 4 weeks (the schedule of events for the additional 4 weeks of treatment is provided in Appendix 17.1).
b. Mandatory for women of childbearing potential; screening: serum screen for pregnancy, Day 29: urine screen for pregnancy.
c. Physical examination to be performed before injection.
d. Blood pressure, pulse rate, temperature, respiratory rate, oxygen saturation; Day 1 and 4: immediately before injection and 15, 30, 45, 60, and 90 minutes and 2, 3, 4, and 6 hours after injection. Day 8, 11, 15, 18, 22, and 25: before and 1, 2, 3, and 4 hours after injection.
e. Hematology, clinical chemistry, and urinalysis (see Section 10.4.6.1 for specific parameters); Day 1: before injection and 6 hours after injection. Day 4 and Day 11: before injection, Day 8, 15, and 22: before injection and 4 hours after injection.
f. Anti-nuclear antibodies (ANA), thyroid-stimulating hormone (TSH), anti-thyroglobulin, rheumatoid factor, anti-smooth muscle antibodies. On Day 15 blood samples will be collected before injection (at the same time as the safety clinical laboratory samples).
g. HIV, HBV, HCV.
h. IgG, IgA, IgM, IgE. On Day 15 blood samples will be collected before injection (at the same time as the safety clinical laboratory samples).
i. Immuno-monitoring: Cytokines: Day 1 and Day 25: before injection and 6 and 24 hours (note: 24 hours not reflected in table) post injection (± 2 hour window for 6 and 24 hour post-injection samples), Day 29 and Week 9 and Week 13 (if applicable). Part of the blood sample collected for immuno-monitoring will also be used for exploratory analysis (sub-study). Exploratory samples: Day 1 (before injection), Day 29, Week 9.
j. PK (RGT100-PEI); Day 1 and Day 25: immediately before injection and 5 minutes, 30 minutes, 2, 4, 6, and 24 hours (note: 24 hours not reflected in table) post injection (±2 hour window for 6- and 24-hour post-injection samples).
k. Documentation of BRAF mutation status in melanoma and other mutations as available at site.
l. Number of lesions (weekly), measurement of cutaneous lesions via caliper (baseline, Day 1, Day 15, Day 29, Week 9, and Week 13) and color photography (baseline, Day 1 before biopsy, Day 8, Day 15, Day 22, Day 29, Week 9, and Week 13).
m. CT/MRI/PET-Scan to document tumor burden according to irRECIST in Solid Tumors; imaging can take place up to 7 days before follow-up visits.
n. Biopsy (preferred excision biopsy) to be taken up to 48 hours before injection of RGT100-PEI. For Day 29 from injected lesion and bystander lesion.
o. After Week 9, only new possibly related AEs will be captured.
p. After Week 9, only further anti-cancer-related medications will be captured.
q. 60-Day follow-up visit only applies to subjects who have not progressed at Week 9 (30 days after last injection).

Table 4: Group B (Liver) Schedule of Assessments

Trial Visits, Investigations, and Interventions	**Screening**	**Treatment Period**				**End of treatment Visit**	**Follow-up Visit**	
	Day -21 to Day-1	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 9**	**Week 13[p]**
		Day 1 **Inj 1**	Day 8 **Inj 2**	Day 15 **Inj 3**	Day 22 **Inj 4**	Day 29 (±3 days)	30 days after last injection (±7 days)	60 days after last injection (±7 days)
RGT100-PEI injection [a]		+	+	+	+			
Informed Consent	+							
Medical History	+							
Eligibility Criteria	+							
Pregnancy Test [b]	+					+		
Electrocardiogram	+					+	+	+
Physical Examination [c]	+	+	+	+	+	+	+	+
ECOG Performance Status	+	+	+	+	+	+	+	+
Vital Signs [d]	+	10+	5+	5+	5+	+	+	+
Overnight observation		+	As deemed appropriate by the investigator					
Height/Weight	+							
Hematology [e]	+	+ +	+ +	+ +	+ +	+	+	+
Clinical Chemistry [e]	+	+ +	+ +	+ +	+ +	+	+	+
Urinalysis [e]	+	+ +	+ +	+ +	+ +	+	+	+
Autoimmunity [f]	+			+		+	+	
Infectious Diseases [g]	+					+	+	
Immunoglobulins [h]	+			+		+	+	
Immuno-monitoring [i]	+	+ + +			+ + +	+	+	+
PK (RGT100-PEI) [j]		**7+**			**7+**			
Mutation Status [k]	+							
Imaging [l]	+						+	+
Biopsy [m]		+			+			

Trial Visits, Investigations, and Interventions	**Screening**	**Treatment Period**				**End of treatment Visit**	**Follow-up Visit**	
	Day -21 to Day-1	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 9**	**Week 13[p]**
		Day 1 **Inj 1**	Day 8 **Inj 2**	Day 15 **Inj 3**	Day 22 **Inj 4**	Day 29 (±3 days)	30 days after last injection (±7 days)	60 days after last injection (±7 days)
Adverse Events [n]	+	+	+	+	+	+	+	+
Concomitant Medication [o]	+	+	+	+	+	+	+	+

Abbreviations: ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; Ig = immunoglobulin; Inj = injection; PK = pharmacokinetics; PR = partial response; SD = stable disease.

a. Subjects with PR or SD at week 9 (30 days after last injection) will be allowed to be treated with a second cycle of RGT100-PEI for an additional 4 weeks (the schedule of events for the additional 4 weeks of treatment is provided in Appendix 17.2).
b. Mandatory for women of childbearing potential; screening: serum screen for pregnancy, Day 29: urine screen for pregnancy
c. Physical examination to be performed before injection.
d. Blood pressure, pulse rate, temperature, respiratory rate, oxygen saturation; Day 1: immediately before injection and 15, 30, 45, 60, and 90 minutes and 2, 3, 4, and 6 hours after injection. Day 8, 15, and 22: before and 1, 2, 3, and 4 hours after injection.
e. Hematology, clinical chemistry, and urinalysis (see Section 10.4.6.1 for specific parameters); Day 1: before injection and 6 hours after injection. Day 8, 15, and 22: before injection and 4 hours after injection.
f. Anti-nuclear antibodies (ANA), thyroid-stimulating hormone (TSH), anti-thyroglobulin, rheumatoid factor, anti-smooth muscle antibodies. On Day 15 blood samples will be collected before injection (at the same time as the safety clinical laboratory samples).
g. HIV, HBV, HCV.
h. IgG, IgA, IgM, IgE. On Day 15 blood samples will be collected before injection (at the same time as the safety clinical laboratory samples).
i. Immuno-monitoring: Cytokines: Day 1 and Day 22: before injection, 6 and 24 hours (note: 24 hours not reflected in table) post injection (± 2 hour window for 6- and 24-hour post-injection samples), Day 29 and Week 9 and Week 13 (if applicable). Part of the blood sample collected for immune-monitoring will also be used for exploratory analysis (sub-study). Exploratory samples: Day 1 (before injection), Day 29, Week 9.
j. PK (RGT100-PEI); Day 1 and Day 22: immediately before injection and 5 minutes, 30 minutes, 2, 4, 6, and 24 hours (note: 24 hours not reflected in table) post injection (± 2 hour window for 6- and 24-hour post-injection samples).
k. Documentation of BRAF mutation status in melanoma and other mutations as available at site.
l. CT/MRI/PET-Scan to document tumor burden according to irRECIST in Solid Tumors; imaging can take place up to 7 days before follow-up visits.
m. Biopsy (preferred excision biopsy) to be taken up to 48 hours before injection of RGT100-PEI.. For Day 22 from injected lesion and non-mandatory from a bystander lesion.
n. After Week 9, only new possibly related AEs will be captured.
o. After Week 9, only further anti-cancer related medications will be captured.
p. 60-Day follow-up visit only applies to subjects who have not progressed at Week 9 (30 days after last injection).

10 EFFICACY, PHARMACOKINETICS, PHARMACODYNAMICS, AND SAFETY VARIABLES

The planned timing of assessments is provided in Section 9: Table 3 for subjects in Group A and Table 4 for subjects in Group B.

10.1 Efficacy Variables

10.1.1 Tumor Assessments by irRECIST

Objective disease response will be measured according to the irRECIST criteria summarized in Appendix 17.3 and will be performed at baseline (within 3 weeks screening period) and after 30 days after the last study drug injection. If there is no progression of disease after 30 days of follow-up the subject will be followed for further 30 days and another tumor assessment will be performed (60 days after last injection).

A clinical and radiological evaluation of malignancies, as judged appropriate by the Investigator, and in line with the protocol, must be performed before starting the study treatment. In Germany only MRI will be used for tumor assessment. The same methods that detected lesions at baseline will be used to follow these lesions throughout the study. To ensure comparability, the radiological assessments used to assess response must be performed using identical techniques.

Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment.

10.1.1.1 Baseline evaluations

These will include clinical and radiological measurements of the extent of disease by CT chest/abdomen/pelvis scan, or MRI scan or PET scan where applicable. Sites should use their routine method. In case a tumor assessment has been performed within the last 4 weeks and images are appropriate for use in this trial and according to irRECIST, such available images can be used and imaging does not need to be repeated. All areas of disease present must be mentioned (even if specific lesions are not going to be followed for response) and the measurements of all measurable lesions must be recorded on the scan reports. Any non-measurable lesions must be stated as being present. All subjects included in the trial will have at least 1 measurable lesion that will be injected with RGT100-PEI, and 1 measurable lesion that will not be injected.

10.1.1.2 Evaluations during treatment and at off-study

Tumor assessment will be repeated as per the schedule of events given, or more frequently if clinically indicated. All lesions measured at baseline must be measured at subsequent tumor assessments, and recorded on the scan reports. All non-measurable lesions noted at baseline must be reported as present or absent.

Investigators must ensure that their radiologists are aware of the requirement to follow up and measure every target lesion mentioned at baseline and comment on the non-target lesions in accordance with irRECIST. All subjects included in the study must be assessed for response to treatment. Each subject will be assigned 1 of the following categories for irRECIST response: CR, PR, SD, PD, NE, early death from malignant disease, early death from toxicity, early death from other cause, or unknown (not assessable, insufficient data). The

applicable overall response category for each visit that includes disease assessment must be recorded in the medical record for inclusion in the appropriate eCRF.

Response duration if applicable will be measured from the time measurement criteria for immune-related (ir) CR/PR are first met until the first date that recurrent or PD is objectively documented. Date of progression is defined as the first day when ir PD is observed.

Should rapid tumor progression occur before the first per protocol timepoint of tumor re-evaluation, the subject will be classified as having early progression. Early death is defined as any death occurring before the first per protocol timepoint of tumor re-evaluation. The responsible Investigator will decide if the cause of death is malignant disease, toxicity or other cause.

Tumor response should be classified as NE, only when it is not possible to classify it under another response category (e.g., when baseline and/or follow-up assessment is not performed or not performed appropriately).

10.1.1.3 Other definitions of outcome

Toxic death: Any death to which drug toxicity is thought to have a major contribution.

Early death: Death during the first 4 weeks of treatment that is not a toxic death.

10.1.2 Tumor Assessments by Photography and Caliper Measurements

Baseline: for subjects in Group A only, the target cutaneous lesions selected for irRECIST evaluation will be measured by caliper and photographed. In addition, the number of cutaneous lesions will be recorded.

For clinical measurements of cutaneous lesions in Group A, documentation by color photography (including size measurement) will be performed at baseline, then every 2 weeks, End-of-Treatment Visit and at follow-up; caliper measurements of lesions will be performed weekly, at the End-of-Treatment Visit, and at follow-up.

10.1.3 Tumor Mutation Assessments

Baseline assessment of BRAF mutation status in melanoma (assumed to be available in the subject chart at the site), human papilloma virus (HPV) in head and neck cancer, mismatch repair status, and PD-1/PD-L1 expression will be assessed. Other mutations deemed appropriate may also be evaluated. The mutation status will be determined in order to evaluate if there is any particular response pattern in the different mutations.

Additional information on tumor mutation status, if and as available at the site, shall be captured in the eCRF.

10.2 Pharmacokinetic Variables

Analysis of plasma samples for the determination of RGT100-PEI will be carried out to assess systemic exposure to locally injected RGT100-PEI. Blood samples for the determination of RGT100-PEI levels will be collected before (maximum 30 minutes) injection and 5 and 30 minutes, 2, 4, 6, and 24 hours post injection on Week 1 Day 1 and on Week 4 Day 25 (Group A) or Week 4 Day 22 (Group B). Windows for sample collection are: 5 minutes (±2 minutes), 30 minutes (±5 minutes), 2 hours (±10 minutes), 4 hours (±30 minutes), 6 hours (±30 minutes), and 24 hours (±2 hours).

Handling and processing details will be outlined in the Laboratory Manual for the RGT100-001 protocol.

10.3 Pharmacodynamic Variables

10.3.1 Immuno-monitoring

10.3.1.1 Cytokines

Blood samples for the analysis of selected cytokines in plasma and other yet to be determined parameters for scientific purposes will be collected. The cytokines will be used as a safety and activity parameter and will be assessed during the dose escalation meetings by the SRC.

Samples for immune-monitoring will be collected at baseline, Week 1 Day 1 and Week 4 Day 25 (Group A) or Week 4 Day 22 (Group B) at 0 minutes, 6 hours and 24 hours post injection, Day 29 and Week 9 as well as Week 13 if applicable).

Additional sample collection, handling, and processing details will be outlined in the Laboratory Manual for the RGT100-001 protocol.

10.3.1.2 Exploratory Analysis

As part of an exploratory sub-study, further information focused on the effect of RGT100-PEI on the immune system will be explored. This may include the induction of immune gene transcripts, the induction of immune cell infiltrates and the histopathological response including the presence of inflammation, proliferation and apoptosis in tumor cells will be explored. Exploratory samples will be taken on Day 1 prior to dosing, Day 29 and Week 9.

As this sub-study will be exploratory in nature, the results will not be presented as part of the main Clinical Study Report.

10.3.2 Tumor Biopsy

Tumor core, punch, or excision biopsies will be taken at baseline (up to 48 hours before the first injection) from lesions chosen for treatment (or a nearby lesion) as deemed appropriate by the Investigator and from 1 previously injected lesion at the day of the last injection and a bystander lesion at Day 22 (Group B) or Day 29 (Group A), respectively. Biopsies are mandatory for all subjects in the trial except the one from a bystander lesion from liver at the end of treatment; this one is optional at the discretion of the Investigator.

Biopsies will be performed in accordance with the study site’s standard procedures. Liver biopsies in Group B will be taken under image guidance, preferably under ultrasound guidance.

Handling and processing details for biopsy evaluation will be outlined in the Laboratory Manual for the RGT100-001 protocol.

10.3.2.1 Optional Genetic Sub-Study

An optional genetic sub-study will be performed on the available biopsy samples. Subjects will be asked to sign a separate consent form to allow for this additional testing to be performed on collected biopsy samples.

10.4 Safety Variables

10.4.1 Adverse Events

10.4.1.1 Adverse Event Definition

An AE is defined as any untoward medical occurrence in a clinical study subject administered a medicinal product which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not it is related to the medicinal (investigational) product. This includes an exacerbation of pre-existing conditions or events, intercurrent illnesses, drug interaction or the significant worsening of the indication under investigation that is not recorded elsewhere in the eCRF under specific efficacy assessments. Anticipated fluctuations of pre-existing conditions, including the disease under study that do not represent a clinically significant exacerbation or worsening need not be considered AEs.

The Investigator will monitor each subject for clinical and laboratory evidence of AEs on a routine basis throughout the study. Should an Investigator become aware of any study drug-related SAEs following this period these must also be reported as stated in Section 10.4.4.3. Adverse event monitoring starts from the time the subject consents to the study until they complete the trial. All reportable AEs will be followed to a satisfactory conclusion. Any reportable drug-related AEs that are unresolved at the follow-up visit are to be followed up by the Investigator until resolution or stabilization.

AEs will be elicited by asking the subject a non-leading question, for example, “Have you experienced any new or changed symptoms since we last asked/since your last visit?” AEs should be reported on the appropriate page of the eCRF.

Each separate AE episode must be recorded. For example, if an AE resolves completely or resolves to baseline and then recurs or worsens again, this must be recorded as a separate AE. For AEs to be considered intermittent, the events must be of similar nature and severity.

Tumor progression is not an AE. However, the medical conditions associated with disease progression must be documented as AE.

10.4.1.2 Onset Date, End Date

If an AE starts during the study but did not end before the final closing (follow-up) visit, the Investigator must make a reasonable effort to establish the outcome and the end date of the AE. If this is not possible (e.g., because the AE is still ongoing) or the subject is lost to follow-up, there will be no end date for the AE.

For all AEs that resolve, resolve with sequelae, or have a fatal outcome, an end date must be provided.

If an AE stops and restarts later, all occurrences have to be recorded separately.

If an AE starts as a non-serious AE and becomes serious at a later point in time, the following applies in regard to the onset date:

The onset date of the AE will reflect the first occurrence of signs and/or symptoms associated with the event, or the date the first diagnosis was made, independent of event seriousness at that time.

10.4.1.3 Assessment of Severity

Severity of AEs refers to the extent to which an AE affects the subject's daily activities. The severity of AEs and toxicities must be graded by the Investigator according to the NCI CTCAE version 4.03. The NCI CTCAE version 4.03 can be found at http://www.oncology.tv/SymptomManagement/NationalCancerInstituteUpdatesCTCAEtov403.aspx.

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

The term "severity" is used to describe the intensity of an AE. This is not the same as "serious". Seriousness, not the severity, serves as the guide for defining regulatory reporting obligations. Notwithstanding this differentiation, AEs of severity grade 4 or 5 (life-threatening of fatal) will meet the criteria for seriousness and will thus qualify as SAEs/irAEs.

If there is a change in severity of an AE, it will be captured as 1 AE, with the highest severity grade recorded.

10.4.1.4 Assessment of Causality

A medically qualified individual (Investigator or his designee) must assess the "relationship" of an AE to RGT100-PEI. The assessment of causality is made using the following categories:

Unrelated:	Clinical event with an incompatible time relationship to study drug administration, and that could be explained by underlying disease or other drugs or chemicals or is incontrovertibly not related to the study drug.
Possible:	Clinical event with a reasonable time relationship to study drug administration, but that could also be explained by concurrent disease or other drugs or chemicals.
Related:	Clinical event with plausible time relationship to study drug administration, and that cannot be explained by concurrent disease or other drugs or chemicals.

The Investigator must endeavor to obtain sufficient information to confirm the causality of the AE (i.e., relation to surgery, study drug, background treatment, other illness, progressive malignancy etc.) and give their opinion of the causal relationship between each AE and study drug. This may require instituting supplementary investigations of significant AEs based on their clinical judgement of the likely causative factors and/or include seeking a further specialist opinion.

Refer to the IB[4] for all nonclinical safety details relating to RGT100-PEI. A copy of the current approved version of the IB must be held in the Site File for reference. Any change or update to the IB during the trial will be made via substantial amendment.

10.4.1.5 Action Taken as a Result of Adverse Event

The action taken as a result of the AE will be documented in the appropriate section of the eCRF as follows:

- None
- Study drug stopped
- Study drug temporarily interrupted
- Concomitant medication changed, stopped
- Medication to treat AE
- Other, specify

10.4.1.6 Outcome of Adverse Event

The final outcome of the AE will be documented in the appropriate section of the eCRF as follows:

- Recovered
- Recovered With Sequelae
- Not Recovered
- Ongoing
- Unknown
- Death

10.4.1.7 Follow-up of Adverse Events

Adverse events should be followed to determine the outcome.

AEs that are serious or considered related or possibly related to study drug or study procedure must be followed up until the event is resolved, resolved with sequelae or until, in the opinion of the Investigator, the event is stabilized, has been fully assessed and does not require further follow-up or determined to be chronic. Details of AE resolution must be documented in the eCRF.

For all SAEs, where important or relevant information is missing, active follow-up should be undertaken.

All other AEs must be followed up by the Investigator until the AE is resolved, resolved with sequelae or the end of the period of observation (= last study visit), whichever comes first.

Subjects should be followed up for 30 days (or 60 days for subject who have not progressed at Day 30) after receiving the last dose of study drug, and any AEs that occur during this time should be reported according to the procedures outlined above. If AEs that are serious and considered related or possibly related to study drug or study procedure are ongoing at the time of subject's last study visit, all attempts should be made to receive the final outcome.

A follow-up report must be completed when an SAE resolves, is unlikely to change, or when additional information becomes available. In case the SAE is a suspected unexpected serious adverse reaction (SUSAR) follow-up information must be provided within the timelines as requested by the INC Research Safety & Pharmacovigilance.

If new or amended information on a reported SAE becomes available, the Investigator can report this on a new SAE form using the completion guidelines. If using the original form to notify further information, all new or amended information need to be initialed and dated in order all changes are clearly identified.

10.4.1.8 Documentation and Reporting of Adverse Events

AEs should be reported and documented in accordance with the procedures outlined below. All AEs occurring during the study must be documented on the relevant eCRF pages. The following data should be documented for each AE:

- Diagnosis or if not available symptoms
- Classification of 'serious' or 'not serious'
- Severity
- Date of first occurrence and date of resolution (if applicable)
- Action taken
- Causal relationship
- Outcome of event

10.4.2 Immune-Related Adverse Events

An irAE, will be considered as a subset of AEs defined as a clinically significant AE of any organ that is associated with study drug exposure, of unknown etiology, and is consistent with an immune-mediated mechanism. Serologic, immunologic, and histologic (biopsy) data may be used to support an irAE diagnosis. Appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxic, or other etiologic causes of the irAE.

10.4.3 Monitoring of Hypersensitivity Reactions, Tumor Spread and Tumor Lysis

Subjects should be observed closely for hypersensitivity reactions and local reactions of the injected tumor site. Blood pressure, pulse, respiration rate, temperature and oxygen saturation should be monitored at the timepoints specified in Table 3 and Table 4. For the first 2 injections the minimum observation period will be 6 hours, and for subsequent injections, the minimum observation period will be 4 hours.

Hypersensitivity to RGT100-PEI is a potential safety risk in general as for any drug. There are no signs from nonclinical work that this may be expected. Hypersensitivity reactions should be dealt with according to local policy. Treatment will be permanently discontinued in the case of grade 3 or 4 hypersensitivity reaction.

A risk of tumor spread from an intratumoral injection has been proposed. However, tumor growth along needle tracks has generally been described only in circumstances where no anti-cancer agent is being administered. In therapeutic trials of intratumoral injection tumor dissemination has not been dose limiting nor reported as a clinically significant event. Injection sites will be carefully monitored throughout subject participation in the trial and any evidence of tumor growth along injection tracks reported as an AE of special interest. So far no data of relevant tumor cell spread have been publically reported from other agents which are intratumorally applied.

Generally there is also the potential risk for tumor lysis, however there are relatively few reports of tumor lysis syndrome in solid tumors. Potential risk factors include disease bulk, aggressive growth, hepatic involvement, and pre-treatment laboratory abnormalities such as elevated lactate dehydrogenase, uric acid, and creatinine. Investigators should be aware of this potential risk and routine measures should be taken for prevention and management.

10.4.4 Serious Adverse Events

10.4.4.1 Serious Adverse Event Definition

A SAE is any AE, regardless of dose, causality or expectedness, that:

- **Results in death:** Death is an outcome of an AE and not an AE in itself.
- **Is life-threatening:** This refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
- **Requires inpatient hospitalization or prolongs existing inpatient hospitalization:** In general, hospitalization signifies that the subject has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered serious. Note: Elective admissions to hospital for subject convenience or for planned procedures or investigations or treatment as specified in this protocol are not SAEs, and do not require SAE reporting. However, if the underlying condition for which hospital treatment or surgery had been planned worsened during the study, the worsening of the condition is to be reported as an SAE.
- **Results in persistent or significant incapacity or disability:** This means a substantial disruption of a person's ability to conduct normal life functions. It does not include experiences of relatively minor medical significance or accidental trauma (e.g., sprained ankle), which do not constitute a substantial disruption.
- **Is a congenital anomaly or birth defect**
- **Is any other medically important event:** Defined as an event that may jeopardize the subject or may require intervention to prevent 1 of the outcomes listed above. Medical and scientific judgement must be exercised in deciding whether an event is serious. Any new primary cancer must be reported as an SAE.

10.4.4.2 Adverse Events not considered serious

The following events do not meet the criteria for SAEs and will therefore not be reported as an SAE:

- Any planned hospitalization before inclusion in the study.
- Admission for elective treatment of a condition unrelated to the studied indication or its treatment.
- Admission as a part of the normal planned treatment or monitoring of the studied indication and not associated with any deterioration in condition.
- Admission for social reasons, without an underlying medical condition, including hospice.
- Disease progression and events which are unequivocally related to disease progression regardless of their outcome and regardless whether they otherwise would fulfil seriousness criteria.

10.4.4.3 Reporting of Serious Adverse Events

SAEs must be reported from signing the informed consent up to and including 30 respectively 60 days after administration of the last dose, whether or not considered to be related to the investigational product.

Any SAE must be reported by the Investigator on a SAE Reporting Form **within 24 hours** of Investigator's awareness to **INC Research Safety & Pharmacovigilnace**:

Email: PPD

Fax: PPD

The 4 minimum criteria for a valid SAE report include:

- Site identifier
- Subject identifier
- Event term
- Study drug

The Investigator should not wait to receive additional information to document fully the event before notification of a SAE. As soon as the 4 minimum mentioned above criteria are fulfilled, the Investigator must report the SAE within 24h of awareness. In addition, all further information requested on the SAE Reporting Form should be completed and sent to INC Research Safety & Pharmacovigilance as soon as possible. If the SAE has not been reported within the specified timeframe, a reason for delay must be provided when sending the SAE Reporting Form.

Where applicable, information from relevant laboratory results, hospital case records, and autopsy reports should be obtained and reported to INC Research Safety & Pharmacovigilance as soon as possible as well.

Instances of death, congenital abnormality, or an event that is of such clinical concern as to influence the overall assessment of safety, if brought to the attention of the Investigator at any time after cessation of study drug administration and linked by the Investigator to this study, should be reported to the study monitor.

Any laboratory values considered clinically significant or meeting grade 4 (NCI CTCAE) or above should be considered AEs and should be captured in the eCRF. Any events reported that also meet the SAE criteria should be reported in line with SAE reporting requirements.

All SAEs reported to INC Research Safety & Pharmacovigilance will be processed according to internal standard operating procedures (SOPs). INC Research Safety & Pharmacovigilance may request additional information for any SAE as judged necessary.

Any SAE occurring at any time after completion of study treatment or after the designated follow-up period that the Investigator consider to be related to any study drug must be reported to the Sponsor/INC Research.

INC Research will promptly notify all participating Investigators, relevant regulatory authorities, and ethics committees of findings that could adversely affect the safety of the subjects, impact on the conduct of the study or alter the Research Ethics Committee (REC) approval/favorable opinion of the study. In addition, INC Research, on behalf of the Sponsor, will expedite the reporting to all concerned Investigators, to the REC, where required, and to the regulatory authorities of all adverse reactions that are both serious and unexpected.

Details of the procedures to be followed if a pregnancy occurs are provided in Section 8.3.4.3.

10.4.5 Unexpected Adverse Reactions

An unexpected adverse reaction is any untoward and unintended response that is related to the administration of the study drug.

All SUSARs will be the subject of expedited reporting. INC Research Safety & Pharmacovigilance shall ensure that all relevant information about a SUSAR that is fatal or life-threatening is reported to the relevant competent authorities and REC within 7 days after knowledge by INC Research and /or the Sponsor of such a case or as defined in local safety reporting requirements and that relevant follow-up information is communicated within an additional 8 days or as defined in local safety reporting requirements. All other SUSARs will be reported to the relevant competent authorities and REC within 15 days after knowledge by INC Research and / or the Sponsor of such a case or as defined in local safety reporting requirements. All Investigators should follow up SUSARs until the event is resolved or until, in the opinion of the Investigator, the event is stabilized or determined to be chronic. Post study SUSARs that occur after the subject has completed the clinical study must also be immediately reported by the Investigator to INC Research and /or the Sponsor.

10.4.6 Laboratory Samples

A central laboratory will be used for evaluation of collective samples from the local study sites.

Details of the timing of clinical laboratory sample collection are provided in in Section 9: Table 3 for subjects in Group A and Table 4 for subjects in Group B.

Details of sample preparation, labelling and dispatch are provided in the sample handling manual for the RGT100-001 protocol. All samples sent to analytical Laboratories will be labelled with the trial code, trial subject number, dosing level and date/time taken. Should a laboratory receive any samples carrying unique subject identifiers, the recipient must immediately obliterate this information and re-label.

Sample collection, handling and processing details will be outlined in the Laboratory Manual for the RGT100-001 protocol.

10.4.6.1 Safety Clinical Laboratory Samples

Hematology: Hemoglobin, hematocrit, white blood cell (total and differential), red blood cell count, platelet count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, international normalized ratio, partial thromboplastin time.

Clinical Chemistry: Creatinine, creatinine clearance (calculated via Cockroft formula), urea, AST, ALT, gamma glutamyltransferase (gamma GT), glutamate dehydrogenase (GLDH), alkaline phosphatase, lactate dehydrogenase (LDH), creatine kinase (CK), total bilirubin, albumin, total protein, sodium, potassium, magnesium, chloride, glucose, uric acid, total cholesterol, triglycerides, calcium, phosphorus, c-reactive protein (CRP).

Urinalysis: Dipstick test (Combur 10) including pH, glucose, ketones, blood and protein

Viral Serology Screen: Serum screening for HBV, HCV, HIV

Pregnancy Screen: At screening: serum screen for pregnancy. At end of treatment (Day 29): urine screen for pregnancy.

Autoimmunity: Development of anti-nuclear antibodies (ANA), thyroid-stimulating hormone (TSH), anti-thyroglobulin, rheumatoid factor and anti-smooth muscle antibodies.

Cytokines: IL6, TNF alpha, IL-12 and IL-1Beta

10.4.6.2 Clinical Reporting of Exploratory Research Assay Results

The results of the RGT100-001 exploratory objectives are trial research assays and are not intended to influence the individual subject's medical care. Findings for exploratory research assays will not be reported routinely in real time to the Investigator except in the unlikely event that the result might be beneficial to the subjects' clinical management. The results of the exploratory objectives will not be reported in the main Clinical Study Report.

10.4.6.3 Trial Sample Retention at End of Study

The Sponsor has the overall responsibility for custodianship of the trial samples. Laboratories are instructed to retain any surplus samples pending instruction from Sponsor on use, storage or destruction. It is possible that new or alternative assays may be of future scientific interest. At the end of the research study any surplus samples may be retained for use in other projects that have received ethical approval. Hence, any surplus study samples may be transferred to a licensed tissue bank where they will be managed in accordance with applicable host institution policies and the Human Tissue Authority requirements.

10.4.7 Other Safety Variables

The timing of each of the following safety variables is provided in Table 3 (Group A Schedule of Events) and Table 4 (Group B Schedule of Assessments).

10.4.7.1 Vital Signs

Vital signs (blood pressure, pulse rate, respiratory rate, temperature, oxygen saturation) will be recorded in a standardized manner, ie, after the subject has rested in the sitting position for 5 minutes.

10.4.7.2 Physical Examination

A physical examination will include the following: examination of general appearance, skin, head, neck (including thyroid), eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system.

Height and weight will be recorded at screening.

10.4.7.3 Electrocardiogram

A 12-lead electrocardiogram (ECG) will be performed in the supine position.

10.4.7.4 ECOG Performance Status

The ECOG performance status will be recorded using the scale provided in Appendix 17.4.

10.5 Safety Review Committee

The Safety Review Committee (SRC) will be composed of but not limited to the Principal Investigator(s), CRO Medical Monitor, Sponsor representative and Investigators and/or representatives from participating sites where possible. Additional experts or persons, both non-voting, may participate as needed.

The SRC will meet when all the subjects in each individual dose cohort have been treated and followed for the specified time, as defined in the study protocol for the safety assessments or sooner, if a significant safety issue arises. Following a thorough review of the available safety information by the SRC from the cohort and any new safety information from the preceding cohorts, the decision will be taken whether or not to increase the dose of the product for the next cohort. The SRC will thus meet after 3-6 subjects in a cohort have completed 4 weeks of treatment to evaluate the safety and initial efficacy data and decide on the next dose escalation. Minutes of these meetings will be taken and the recommendation from the SRC in relation to the dose increment will be recorded. Details of the organization, content and procedures of these dose escalation meetings and functioning of the SRC will be described in the SRC Charter.

Bi-Weekly Safety Calls will be held in addition to Dose Escalation Meetings during the dose escalation phase.INC Research will organize the Bi-Weekly Safety Calls and the Dose Escalation /SRC Meetings. The Bi-Weekly safety calls will commence approximately two weeks after the first subject is dosed at the first site. The SRC/Dose Escalation Meetings will be held upon completion of at least 3 evaluable subjects on each dose level (and additionally as required and agreed) during the pre-scheduled bi-weekly calls - ie the Bi-Weekly Safety Calls will serve as Dose Escalation Meetings when appropriate. Selected members of Rigontec and INCR teams will join during the Bi-Weekly Safety Calls. During the Dose Escalation Meetings, the aim is for the full SRC to be present. It is critical for the investigator of an active site to be in attendance during these safety meetings. The timing for the Dose Escalation Meetings will be fixed when the 3rd subject in a cohort has received their first

dose, and as appropriate depending on the SRC agreement on further subjects enrolled in cohort(s), if any. The meeting agenda, as well as listings of the safety data of the subjects, will be distributed prior to the call.

10.6 Appropriateness of Measurements

The efficacy, safety and pharmacodynamic assessments planned for this study are widely used and generally recognized as reliable, accurate and relevant to the disease condition.

11 STATISTICAL METHODS

11.1 Statistical and Analytical Plans

A detailed statistical analysis plan will be finalized before any analysis is undertaken. The analysis plan will be written in accordance with the current SOPs and will be finalized and agreed by the Trial Statistician, the Lead Investigator, and the Sponsor.

11.1.1 Procedures for Reporting Any Deviation(s) from the Original Statistical Plan

Any deviations from the analyses planned in the protocol will be detailed in the Statistical Analysis Plan (SAP) and deviations from the original statistical plan will be captured in the Clinical Study Report.

11.1.2 Datasets or Populations Analyzed

All subjects enrolled in the study will be accounted for. The number of subjects who were NE, who died, or who withdrew before treatment began will be recorded. All subjects who received at least 1 dose of the study drug will be included in the analysis for toxicity.

Two subject populations will be used for the analyses:

- The safety population will consist of all enrolled subjects who received at least 1 dose of RGT100-PEI.
- The evaluable population will consist of all enrolled subjects who complete Week 4 or withdraw early for experiencing a DLT.

11.1.2.1 Dose Escalation

All evaluable subjects will be analyzed for dose escalation, which are those completing Week 4 or those withdrawing early for experiencing a DLT. Number and percentage of subjects with and without DLT will be presented according to DL.

11.1.2.2 Dose Expansion

All subjects who received at least 1 dose of the study drug will be included in the analysis.

11.1.3 Demographic and Other Baseline Characteristics

11.1.3.1 Demographic data

Characteristics of the subjects, including medical history and disease characteristics at baseline, will be listed for each subject and summarized for the total sample and by DL and cohort. Medical history findings will be summarized using Medical Dictionary for Regulatory Activities (MedDRA) terms. Reasons for discontinuation of investigational product will be listed including the study day of treatment discontinuation and will be summarized by DL and group.

11.1.3.2 Exposure

Exposure to investigational product (i.e., total amount of study drug received) will be listed for all subjects by group and DL.

Total exposure (date of last dose minus date of first dose+1) and total time on study (date of discontinuation minus date of first dose+1) will be summarized by the following: mean, standard deviation, minimum, maximum, median, and number of observations. In addition, the number and percentage of subjects with at least 1 dose interruption/dose delay will be presented separately for each DL and group.

Relative dose intensity and percentage intended dose for RGT100-PEI will be derived and summarized by the following: median, minimum, maximum, 75th percentile, 25th percentile and number of observations. Relative dose intensity is the percentage of actual dose intensity delivered relative to the intended dose intensity through treatment discontinuation. Percentage intended dose is the percentage of the actual dose delivered relative to the intended dose through progression.

11.1.4 Efficacy Variables

11.1.4.1 Tumor response

Tumor response will be assessed to support the secondary objectives of the study. Tumor response data will be listed and summarized by cohort and visit (that includes disease assessment) using the following response categories: CR, PR, SD, PD, NE, early death from malignant disease, early death from toxicity, early death from other cause, or unknown (not assessable, insufficient data). The results of lesion measurements, changes in size and supportive laboratory parameters will be analyzed and summarized. Evaluations at screening and at Day 1 will serve as baseline values. Changes from baseline for continuous variables and shift tables for categorical variables will be presented by cohort and by visit of tumor assessment. Descriptive statistical methods as outlined in detail in the SAP will be used.

Objective response rate as defined by achieving CR or PR will be presented by percentage rates and 95% confidence intervals assuming an exact binomial distribution (Clopper–Pearson method). Fisher's exact test (univariate analyses) and logistic regression may be used to compare the response rates of the different cohorts.

The duration of response (DR) will only be calculated for subjects who have an objective response. The DR is calculated from the date of the first documented response until the date of progression or death; subjects who continue to respond at the date of data cut-off (last subject last visit) will be censored at their last known date. Summaries of DR (n, medians, quartiles) will be provided.

11.1.4.2 Tumor Mutation Assessments

Tumor response data will be summarized by mutation status. Logistic regression analyses may be used to test potential predictive factors (BRAF if available from the site, human papilloma virus, mismatch repair status, PD-1/PD-L1 expression) for overall response.

11.1.4.3 Pharmacokinetics

Standard PK parameters will be calculated by use of validated PK software. The definitions of the PK parameters and the equations used to calculate them will be provided in the SAP. A listing of PK sampling dates and times will be provided. Plasma concentrations of RGT100-PEI will be summarized by nominal sample time. Plasma concentrations and derived PK parameters for RGT100-PEI will be summarized by cohort using descriptive statistics (n, geometric mean, arithmetic mean, standard deviation and coefficient of variation, minimum, maximum). Values below the limit of quantification will be set to zero but excluded from the calculation of the geometric mean.

No adjustments for pre-planned multiple comparisons will be made.

11.1.4.4 Pharmacodynamics

Pharmacodynamic variables based on blood samples and biopsy samples will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum, maximum) by cohort. Summaries of change from baseline and percent change from baseline will also be presented for pharmacodynamic variables by cohort.

11.1.5 Methods of Analysis

Descriptive and inferential statistics will be used to summarize the data. Continuous variables will be summarized by n, mean, standard deviation, median, minimum and maximum values. Categorical variables will be summarized by subject counts and related percentages.

On an exploratory basis, common statistical methodology for response rates and survival time analysis may be applied. Counts and percentages, with their corresponding exact 95% confidence interval, will be calculated for the binomial endpoints (i.e., response rate).

Logistic regression analyses may be used to test potential predictive factors for overall response. A more detailed description of the statistical methods will be provided in a separate SAP.

11.1.6 Safety Variables

The DLTs occurring during Phase 1 will serve as the basis for the selection of the RP2D. Number and percentage of subjects with and without DLT will be presented according to DL and group.

Safety will be assessed on the basis of AE reports, clinical laboratory data, vital signs, ECGs, physical examinations and ECOG performance status. All safety summaries will be presented by total and DL for Phase 1, and total and group for Phase 2. The number of subjects experiencing each AE will be summarized by the MedDRA system organ class, MedDRA preferred term, and NCI CTCAE grade. In addition, the incidence, severity, seriousness, and relationship to study treatment of AEs will be tabulated. Serious adverse events and irAEs will be summarized separately.

Hematology, clinical chemistry, autoimmunity, infectious disease, vital signs, and ECG data will be listed individually by subject and suitably summarized. For all laboratory variables, which are included in the current version of NCI CTCAE, the CTCAE grade will be calculated. Summary statistics of mean, median, standard deviation, minimum, maximum and

number of observations will be used and changes from baseline will also be presented. For urinalysis parameters, any qualitative assessments will be summarized for all subjects using the number of subjects with results of negative, trace or positive. For laboratory data, abnormal values will be flagged in the data listings.

Adverse events will be listed individually by subject, DL, and group. Adverse event listings will also be presented by subject, system organ class, and preferred term.

Details of any deaths will be listed for all subjects. Deaths due to the study disease, toxic deaths and early deaths as well as the date of death as reported on the Death Form will be listed and summarized by DL and group.

11.1.7 Sub-group Analysis

No subgroup analysis will be performed for the dose escalation part of the trial. Clinical safety and activity will be assessed in the escalation phase in order to decide on the indications for the expansion groups.

A safety report will be completed at the end of the dose escalation phase.

A SRC will meet to review the safety results from a given cohort before escalating to the next dose in a new cohort.

11.1.8 Handling of Missing Data

Missing data will not be replaced. Analyses will be performed considering all data observed for the respective analysis sets. For missing AEs and concomitant medication start and end dates the followings rules will be applied:

Partial/missing start date:

- Missing day: Impute the first of the month unless month is same as month of first dose of study drug then impute first dose date
- Missing day and month: impute 1st January unless year is the same as first dose date then impute first dose date
- Completely missing: impute first dose date unless the end date suggests it could have started before this in which case impute the 1st January of the same year as the end date.

Partial/missing end date:

- Missing day: Impute the last day of the month unless month is same as month of last dose of study drug then impute last dose date
- Missing day and month: impute 31st December unless year is the same as last dose date then impute last dose date

11.2 Determination of Sample Size

11.2.1 Dose Escalation Phase

Between 9 and 24 evaluable subjects will be recruited to each group (Group A and Group B) in the dose escalation phase of the trial in a 3 + 3 design to establish a RP2D as explained in earlier sections of the protocol. Each dose escalation needs to be agreed to by the SRC based on the definitions in this protocol.

11.2.2 Expansion Phase

Up to 60 evaluable subjects will be recruited to the expansion phase of the trial at the RP2D to further delineate safety and clinical activity of RGT100-PEI. There will be up to 4 groups of 15 subjects each with selected tumor indications. No formal power calculations were performed to obtain the suggested sample size. During further therapy, subjects will continue to be assessed for safety and tolerance, as well as for preliminary evidence of anti-tumor efficacy of RGT100-PEI.

Hence the number of subjects has been based on the desire to obtain adequate safety, tolerability, anti-tumor activity, PK, data while exposing as few subjects as possible to the investigational product and procedures.

11.3 Protocol Deviations

Subject safety is the primary concern of the Investigator, and this protocol recognizes that in some instances, not all protocol-specified details may be followed exactly. If it is deemed that the Investigator is acting urgently in the subject's best interest (e.g., in an emergency) at a given time, investigations or procedures may be omitted or conducted outside the specified timeframes. In the event of the need to significantly deviate from the protocol for any deviations that could potentially impact the subject's safety or the efficacy of study drug, the Investigator should contact the CRO or Sponsor as soon as possible to permit a decision as to whether or not the subject is to continue in the study, if possible. These deviations will be reported to the ethics committee in line with the sites ethics reporting requirements

The nature and reasons for any protocol deviation will be recorded in the subject's eCRF. Additional investigations obtained outside the protocol will not be considered a protocol deviation, as these may be required by the subject's usual treating doctors (e.g., a chest X-ray or urine test in the evaluation of an infection by the subject's local doctor), or in the event that the subject requires attendance at an emergency department for reasons unrelated to the study.

12 QUALITY ASSURANCE AND QUALITY CONTROL

12.1 Audit and Inspection

All aspects of the study conduct may be subject to internal or external quality assurance audit to ensure compliance with the protocol, GCP requirements, and other applicable regulation or standards. It may also be subject to a regulatory inspection. Such audits or inspections may occur at any time during or after the completion of the study. Investigators and their host institution(s) should understand that it is necessary to allow auditors/inspectors direct access to all relevant documents, study facilities and to allocate their time and the time of their staff to facilitate the audit or inspection visit. Anyone receiving notification of a regulatory inspection that will (or is likely to) involve this trial must inform the CRO without delay.

12.2 Risk Assessment

A risk assessment and a monitoring plan will be prepared before the study opens and will be reviewed throughout the study if necessary in the light of significant changes while the study is ongoing or in response to outcomes from monitoring activities. Monitoring plans will be amended as appropriate.

12.3 Monitoring

Regular monitoring will be performed according to the monitoring plan. Data will be evaluated for compliance with the protocol, completeness and accuracy. The Investigator and institutions involved in the study will permit study-related monitoring and provide direct on-site access to all study records and facilities if required. They will provide adequate time and space for the completion of monitoring activities.

Study sites will also be monitored centrally by checking incoming data for compliance with the protocol, consistency, completeness and timing. The case report data will be validated using appropriate set criteria, range and verification checks. The study site must resolve all data queries in a timely manner. All queries relating to key outcome and safety data and any requiring further clarification will be referred back to the study site for resolution. For other non-critical data items, CRO staff may resolve data queries centrally providing the correct answer is clear. Such changes will be clearly identified in the eCRF and the study site informed.

Study sites will also be monitored remotely and/or by site visit as necessary to ensure their proper conduct of the trial. CRO staff will be in regular contact with site personnel to check on progress and deal with any queries that they may have. The Investigator is expected to action any points highlighted through monitoring and must ensure that corrective and preventative measures are put into place as necessary to achieve satisfactory compliance.

12.4 Data Management and Coding

The CRO will be responsible for activities associated with the data management of this study. This will include setting up a relevant database and data transfer mechanisms, along with appropriate validation of data and resolution of queries. Data generated within this clinical study will be handled according to the relevant SOPs of the data management and biostatistics departments of the CRO.

Study sites will enter data directly into an electronic data capture system by completing the eCRF via a secure internet connection. Data entered into the eCRF must be verifiable against source documents at the study site. Data to be recorded directly on the eCRF will be identified and the eCRF will be considered the source document. Any changes to the data entered into the electronic data capture system will be recorded in the audit trail and will be United States Food and Drug Administration (FDA) CFR 21 Part 11 compliant. The CRO will provide sites with instructions and a video link for training purposes.

Medical coding will use MedDRA for concomitant diseases and AEs and the World Health Organization Drug Dictionary for medications.

Missing or inconsistent data will be queried via an electronic query raised in the clinical database to the Investigator for clarification. Subsequent modifications to the data will be made by the site directly in the clinical database.

The participants will be identified by a unique trial specific number code in the database. The name and any other identifying detail will NOT be included in any trial data electronic file.

The Investigator and study site staff will ensure that data collected on each subject is recorded in the eCRF as accurately and completely as possible. All appropriate laboratory data, summary reports and Investigator observations will be entered into the eCRF from the relevant source data held in the site medical record(s). It is important to ensure that:

- The relevant eCRFs are completed in a timely fashion.
- All eCRF data are verifiable in the source documentation or the discrepancies must be explained.
- eCRF sections are completed in a timely fashion, as close to the visit or event being recorded as possible.
- Data queries are resolved and documented by authorized study staff. The reason for the change or correction should be given where appropriate.
- As much data as possible is entered and cleaned in preparation for each study database lock point.

The above considerations also apply to subjects who are withdrawn early. If a subject withdraws from the study, the reason must be noted on the appropriate form and the subject must be followed up as per protocol.

12.5 Clinical Study Report

All clinical data will be presented at the end of the study as data listings. These will be monitored to confirm the lists accurately represent the data collected during the course of the study. The trial data will then be locked and a final data listing produced. The Clinical Study Report will be based on the final data listings. The locked trial data may then be used for analysis and publication.

13 RECORDS AND SUPPLIES

13.1 Drug Accountability

On receipt of the study drug, the Investigator (or designee) will conduct an inventory of the supplies and verify that study drug supplies are received intact and in the correct amounts before completing a supplies receipt. The Investigator will retain a copy of this receipt at the study site and return the original receipt to the study monitor. The monitor may check the study supplies at each study site at any time during the study.

The RGT100-PEI finished drug product should be stored safely, in a lockable location frozen at -20ºC (±5ºC) in an upright position. The vials should be at room temperature at the time of injection. The vials of RGT100-PEI are for single use only and any unused dispersion should remain in the used vial for drug accountability purposes.

It is the responsibility of the study monitor to ensure that the Investigator (or designee) has correctly documented the amount of the study drug received, dispensed, and returned on the dispensing log that will be provided. A full drug accountability log will be maintained at the study site at all times. The study monitor will perform a final inventory of study drug and final drug accountability check at least before closure of the study site. All discrepancies must be accounted for and documented.

The destruction of used and partially used vials will be undertaken at the site according to local hospital policy. The procedure for destruction of unused vials should be adequate and typically via incineration

13.2 Financing and Insurance

13.2.1 Sponsorship

The Sponsor will provide written confirmation of Sponsorship and authorize the trial commencement once satisfied that all arrangements and approvals for the proper conduct of the trial are in place.

13.2.2 Indemnity

Insurance of this study will be outlined in a separate agreement between CRO and the Sponsor.

13.2.3 Contracts/Agreements

This trial is subject to the Sponsor’s policy requiring that written contracts/agreements are agreed formally by the participating bodies as appropriate. A Clinical Trial Agreement will be placed between the Sponsor or CRO on behalf of the Sponsor and the participating organizations before site activation. The Sponsor or CRO on behalf of the Sponsor will also set up written agreements with any other external third parties involved in the conduct of the trial as appropriate.

Each participating site will follow local regulations and policy on reimbursement of travel expenses of subjects.

14 ETHICS

14.1 Independent Ethics Committee

Before initiation of the study at each study site, the protocol, subject information sheet, consent form and any other information that will be presented to potential trial subjects (e.g., advertisements or information that supports or supplements the informed consent) will be reviewed and approved by an appropriately constituted REC. Principal Investigators will be approved by the REC. Written approval of the study and all relevant study information must be obtained before the study site can be initiated or the study drug is released to the Investigator. Any necessary extensions or renewals of REC approval must be obtained for changes to the study such as amendments to the protocol, the subject information sheet and consent form or other study documentation. The written approval of the REC together with the approved subject information sheet and consent form must be filed in the study files on site.

The Investigator will report promptly to the REC any new information that may adversely affect the safety of the subjects or the conduct of the study as required. The Investigator will submit written summaries of the study status to the REC as required. On completion of the study, the REC will be notified that the study has ended.

14.2 Regulatory Authorities

Relevant study documentation will be submitted to the regulatory authorities of the participating countries and local authorities as required, according to local/national requirements, for review and approval before the beginning of the study. On completion of the study, the regulatory authorities will be notified that the study has ended.

14.3 Ethical Conduct of the Study

The Investigator(s) and all parties involved in this study should conduct the study in adherence to the ethical principles based on the Declaration of Helsinki, GCP, ICH guidelines, and the applicable national and local laws and regulatory requirements.

14.4 Local Research Governance

Investigators are responsible for ensuring they obtain local management agreement to conduct the trial in accordance with local arrangements and policies.

14.5 Informed Consent

The Investigator is responsible for ensuring that no subject undergoes any study-related examination or activity before that subject has given written informed consent to participate in the study.

Potential participants will be given a current, approved version of the subject information sheet and consent form. They will also receive clear verbal information about the study detailing no less than: the nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be explained that they will be free to withdraw from the study at any time, for any reason, without prejudice to future care, and with no obligation to give a reason for withdrawal. They will have sufficient time to

consider the information provided and the opportunity to question the Investigator, their GP or other independent parties before deciding whether to participate.

The Investigator who obtains consent must be suitably qualified and experienced. All delegates must be authorized by the Principal Investigator to obtain consent. The Investigator is responsible for ensuring that the trial consent procedures comply with current applicable GCP, regulatory and ethical requirements. Informed consent discussions and outcomes must be well documented in the medical record. The Investigator must be satisfied that the subject has made an informed decision before taking consent. The subject and the Investigator must personally sign and date the current approved version of the informed consent form according to the sites respective countries regulations. A copy of the information and signed consent form will be given to the participant. The original signed form will be retained at the trial site, with copies held in both the medical record and Investigator Site File (ideally the original if local policy permits).

If new information becomes available that may be relevant to the subject's willingness to continue participation in the study, a new subject information sheet and consent form will be approved by the REC (and regulatory authorities, if required). The study subjects will be informed about this new information and re-consent will be obtained.

Contraceptive/pregnancy counselling

All participants must be advised on the need to use reliable methods of contraception during the study. The advice should include:

1. The acceptable methods, including: male or female sterilization, implants, injectable, combined oral contraceptives, some intrauterine devices, and abstinence (i.e., refraining from heterosexual intercourse for the entire period of risk [during treatment and for 6 months after]). Periodic abstinence is not acceptable (calendar, symptothermal, post-ovulation methods), nor is the withdrawal method (coitus interruptus). The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.
2. The recommendation that a barrier method should be used in addition to another form of contraception.
3. Males and females should continue to take these precautions for a minimum 6 months after the last dose of study drug.
4. That any pregnancy (also applies to females partners of male trial subjects) occurring within 6 months of the last administration of study drug will be followed up and the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) will be reported and followed up even if the participant is discontinued from the study.

14.6 Subject Confidentiality

Personal data recorded on all documents will be regarded as confidential, and to preserve each subject's anonymity, only their initials and date of birth will be recorded on the eCRFs.

The Investigator site must maintain the subject's anonymity in all communications and reports related to the research. The Investigator site team must keep a separate log of enrolled subjects' personal identification details as necessary to enable them to be tracked. These documents must be retained securely, in strict confidence. They form part of the Investigator Site File and are not to be released externally.

Monitors, auditors, and other authorized agents of the Sponsor and/or its designee, the REC(s) approving this research, regulatory authorities, as well as that of any other applicable agencies, will be granted direct access to the study subjects' original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the subjects to the extent permitted by the law and regulations. In any presentations of the results of this study or in publications, the subjects' identity will remain confidential.

14.7 Protocol Amendments

Amendments are changes made to the research following initial approval. A 'substantial amendment' is an amendment to the terms of the responsible authority application (if applicable), the REC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the subjects of the trial;
- The scientific value of the trial;
- The conduct or management of the trial; or
- The quality or safety of the investigational medicinal product(s) used in the trial.

Non-substantial amendments are those where the change(s) involve only minor logistical or administrative aspects of the study.

All amendments will be generated and managed according to the CRO's SOPs to ensure compliance with applicable regulations and other requirements. Written confirmation of all applicable REC, regulatory and local approvals must be in place before implementation by Investigators. The only exceptions are for changes necessary to eliminate an immediate hazard to study subjects (see below).

It is the Investigator's responsibility to update subjects (or their authorized representatives, if applicable) whenever new information (in nature or severity) becomes available that might affect the subject's willingness to continue in the trial. The Investigator must ensure this is documented in the subject's medical notes and the subject is re-consented if appropriate.

14.8 Urgent Safety Measures

The Sponsor or Investigator may take appropriate urgent safety measures to protect trial participants from any immediate hazard to their health or safety. Urgent safety measures may be taken without prior authorization. The trial may continue with the urgent safety measures in place. The Investigator must inform the CRO and / or Sponsor immediately if the study site initiates an urgent safety measure:

The notification must include:

- Date of the urgent safety measure;
- Who took the decision; and
- Why the action was taken.

The Investigator will provide any other information that may be required to enable the CRO to report and manage the urgent safety measure in accordance with the current regulatory and ethical requirements for expedited reporting and close out. The CRO will follow written procedures to implement the changes accordingly.

14.9 Temporary Halt

The Sponsor and Investigators reserve the right to place recruitment to this protocol on hold for short periods for administrative reasons or to declare a temporary halt. A temporary halt is defined as a formal decision to:

- Interrupt the treatment of subjects already in the trial for safety reasons;
- Stop recruitment on safety grounds; or
- Stop recruitment for any other reason(s) considered to meet the substantial amendment criteria, including possible impact on the feasibility of completing the trial in a timely manner.

The CRO will report the temporary halt via an expedited substantial amendment procedure to authorities as required. The trial may not restart after a temporary halt until a further substantial amendment to re-open is in place. If it is decided not to restart the trial this will be reported to authorities as an early termination as required.

14.9.1 Serious Breaches

The CRO and/or the Sponsor should notify any "serious breaches" to the appropriate authorities within 7 days of CRO and/or the Sponsor becoming aware of the breach. A serious breach is defined as “A breach of GCP or the trial protocol which is likely to effect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial; or
- the scientific value of the trial”

Investigators must notify the CRO upon awareness within 1 working day if any serious breach of GCP is suspected. The CRO will review the event and, if appropriate will report a serious breach to all required agencies and authorities within 7 days of the CRO becoming aware of the breach or sooner as required by local regulation.

14.9.2 Trial Reports

Where appropriate, the CRO will be asked to submit Development Safety Update Reports.

This protocol will comply with all current applicable Regulatory Authority, Ethics Committee, and Sponsor reporting requirements.

The CRO will determine which reports need to be circulated to Principal Investigators and other interested parties. Study sites are responsible for forwarding trial reports they receive to their local organizations as required.

15 REPORTING AND PUBLICATION, INCLUDING ARCHIVING

During the clinical trial and after trial closure the Investigator must maintain adequate and accurate records to enable the conduct of the clinical trial and the quality of the research data to be evaluated and verified. Essential documents are those documents that individually and collectively permit evaluation of the study and quality of the data produced. All essential documents must be stored by the Investigator, in such a way that ensures that they are readily available upon request, for the period required by ICH GCP guidelines and national legislation (whichever is longer) or for a longer period if needed. The medical files of trial subjects must be retained in accordance with applicable national legislation and the host institution policy. Generally archiving of essential documents is planned for 15 years after the trial was completed if not specified otherwise. Retention and storage of laboratory records for clinical trial samples must also follow these guidelines. It is the responsibility of the Sponsor to inform the study site when these documents no longer need to be retained. The Investigator must contact the Sponsor before destroying any study-related documentation.

Retention and storage of central laboratory records supporting PK or pharmacodynamic endpoints and the disposition of samples donated via the trial must also comply with applicable legislation and Sponsor requirements.

The Sponsor will retain ownership of all data arising from the trial. The Sponsor must review and approve any results of the study or abstracts for professional meetings prepared by the Investigator(s). Published data must not compromise the objectives of the study. Data from individual study sites in multicenter studies must not be published separately.

16 REFERENCES

1. Globocan 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx
2. Schlee M. Master Sensors of Pathogenic RNA-RIG-I like Receptors. Immunobiology. 2013;218:1322-35.
3. Besch R, Poeck H, Hohenauer T, Senft D, Häcker G, Berking C, Hornung V, Endres S, Ruzicka T, Rothenfusser S, Hartmann G. Proapoptotic signaling induced by RIG-I and MDA-5 results in type I interferon-independent apoptosis in human melanoma cells. J Clin Invest. 2009;119(8):2399-411.
4. Rigontec GmbH RGT100 Investigator Brochure, 15 Sept 2016.
5. Note for Guidance on Good Clinical Practice (CPMP/GCP/135/95) and Note for Guidance on Good Clinical Practice (CPMP/GCP/135/95) annotated with Therapeutic Goods Administration (TGA) comments (DSEB, July 2000).
6. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412-20.
7. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228-47.
8. Bohnsack OL, Ludajic K, Hoos A. Adaptation of the immune-related response criteria: irRECIST. ESMO 2014 Abstract 4958. https://www.parexel.com/files/7214/2313/4150/Adaptation_of_the_Immune_Related_Response_Criteria_irRECIST_online.pdf.

17 APPENDICES

17.1 Group A (Cutaneous) Schedule of Assessments Extended Treatment Period

Trial Visits, Investigations and Interventions	Treatment Period								End-of-Treatment Visit	Follow up Visit
	Week 9[k]		Week 10		Week 11		Week 12		Week 13	Week 17
	Inj 9	Inj 10	Inj 11	Inj 12	Inj 13	Inj 14	Inj 15	Inj 16		30 days after last injection (±7 days)
RGT100 injection	+	+	+	+	+	+	+	+		
Pregnancy Test [a]	+								+	
Electrocardiogram	+								+	
Physical Examination	+		+		+		+		+	+
ECOG Performance Status	+		+		+		+		+	+
Vital Signs [b]	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+	+
Overnight observation	As deemed appropriate by the investigator									
Hematology [c]	+		+		+		+		+	+
Clinical Chemistry [c]	+		+		+		+		+	+
Urinalysis [c]	+		+		+		+		+	+
Autoimmunity [d]									+	
Infectious Diseases [e]									+	
Immunoglobulins [f]									+	
Immuno-monitoring [g]									+	
Tumor Assessment [h]	+		+		+		+		+	+
Imaging [i]										+
Biopsy [j]									(+)	
Adverse Events	+	+	+	+	+	+	+	+	+	+
Concomitant Medication	+	+	+	+	+	+	+	+	+	+

Abbreviations: ECOG = Eastern Cooperative Oncology Group

a. Mandatory for women of child bearing potential (urinary screen)
b. Blood pressure, pulse rate, temperature, respiratory rate, oxygen saturation; immediately before injection and hourly up to 2 hours after each injection.
c. Hematology, clinical chemistry, and urinalysis (see Section 10.4.6.1 for specific parameters). Before injection.

d. ANA, TSH, anti-thyroglobulin, rheumatoid factor, anti-smooth muscle antibodies
e. HIV, HBV, HCV
f. IgG, IgA, IgM, IgE
g. Immuno-monitoring: Cytokines and exploratory samples
h. Number of lesions (weekly), measurement of cutaneous lesions via caliper, photography of lesions every other week
i. CT/MRI/PET-Scan to document tumor burden according to irRECIST; imaging can take place up to 7 days before follow-up visits.
j. Biopsy (preferred excision biopsy) is optional.
k. Can be week 9 or after the next follow up at the discretion of the investigator

17.2 Group B (Liver Tumors) Schedule of Assessments Extended Treatment Period

Trial Visits, Investigations and Interventions	Treatment Period				End-of-Treatment Visit	Follow up Visit
	Week 9[i]	Week 10	Week 11	Week 12	Week 13	Week 17
	Inj 5	Inj 6	Inj 7	Inj 8		30 days after last injection (±7 days)
RGT100 injection	+	+	+	+		
Pregnancy Test [a]	+				+	
Electrocardiogram					+	
Physical Examination	+	+	+	+	+	+
ECOG Performance Status	+	+	+	+	+	+
Vital Signs [b]	+ + +	+ + +	+ + +	+ + +	+	+
Overnight observation	As deemed appropriate by the investigator					
Hematology [c]	+	+	+	+	+	+
Clinical Chemistry [c]	+	+	+	+	+	+
Urinalysis [c]	+	+	+	+	+	+
Autoimmunity [d]					+	
Infectious Diseases [e]					+	
Immunoglobulins [f]					+	
Immuno-monitoring [g]					+	
Imaging [h]						+
Biopsy					(+)	
Adverse Events	+	+	+	+	+	+
Concomitant Medication	+	+	+	+	+	+

Abbreviations: ECOG = Eastern Cooperative Oncology Group

a. Mandatory for women of child bearing potential (urinary screen)
b. Blood pressure, pulse rate, temperature, respiratory rate, oxygen saturation; immediately before injection and hourly up to 2 hours after each injection.
c. Hematology, clinical chemistry, and urinalysis (see Section 10.4.6.1 for specific parameters). Before injection.
d. ANA, TSH, anti-thyroglobulin, rheumatoid factor, anti-smooth muscle antibodies
e. HIV, HBV, HCV
f. IgG, IgA, IgM, IgE
g. Immuno-monitoring: Cytokines and exploratory samples

h. CT/MRI/PET-Scan to document tumor burden according to irRECIST in Solid Tumors; imaging can take place up to 7 days before follow-up visits.
i. Can be week 9 or after the next follow up.

17.3 ECOG Performance Scale

Activity Performance Description	**Score**
Fully active, able to carry on all pre-disease performance without restriction.	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	1
Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	2
Capable of only limited self-care. Confined to bed or chair more than 50% of waking hours.	3
Completely disabled. Cannot carry out any self-care. Totally confined to bed or chair.	4
Death	5

17.4 Measurement of Disease – irRECIST

Objective tumor response and time of progression will be measured according to the adapted irRECIST. In 2009, immune-related response criteria [6] were composed when RECIST [7] were found not to provide a complete assessment of immunotherapeutic agents. Immunotherapeutic agents produce anti-tumor effects very different from the ones caused by cytotoxic agents. In 2014, immune-related response criteria and RECIST were combined to form the irRECIST and presented at ESMO10[8].

The following paragraphs are a quick reference to the irRECIST. The complete criteria were published online and can be reached through the following link:

https://www.parexel.com/files/7214/2313/4150/Adaptation_of_the_Immune_Related_Response_Criteria_irRECIST_online.pdf

Baseline:

Measurable Lesion Definition and Target Lesion Selection

- Follow the definitions from RECIST 1.1.
- Up to 5 target lesions may be selected at baseline.
- Measurable lesions must be accurately measured in at least 1 dimension with a minimum size of:
    - 10 mm in the longest diameter by CT or MRI scan (or no less than double the slice thickness) for non-nodal lesions and ≥15 mm in short axis for nodal lesions.
    - 10 mm caliper measurement by clinical exam.
    - 20 mm by chest X-ray.
    - Regardless of the imaging modality blastic bone lesions will not be selected as target lesions. Lytic or mixed lytic-blastic lesions with a measurable soft tissue component ≥ 10 mm can be selected as target lesions.
    - Brain lesions detected on brain scans can be considered as both target or non-target lesions.
    - Lesions that are partially cystic or necrotic can be selected as target lesions. The longest diameter of such a lesion will be added to the total measured tumor burden (TMTB) of all target lesions at baseline. If other lesions with a non-liquid/non-necrotic component are present, those should be preferred.
    - During target lesion selection the radiologist will consider information on the anatomical sites of previous intervention (e.g., previous irradiation, RF-ablation, transcatheter arterial chemoembolization, surgery). Lesions undergoing prior intervention will not be selected as target lesions unless there has been a demonstration of progress in the lesion.

Non-target Lesions

- Follow the definitions from RECIST 1.1.
- There is no limit to the number of non-target lesions that can be recorded at baseline.
- Non-target lesions will include:
    - Measurable lesions not selected as target lesions.

- All sites of non-measurable disease, such as neoplastic masses that are too small to measure because their longest uninterrupted diameter is < 10 mm (or < 2 times the axial slice thickness).
    - The longest perpendicular diameter is ≥ 10 mm and < 15 mm.
- Other types of lesions that are confidently felt to represent neoplastic tissue, but are difficult to measure in a reproducible manner. These include bone metastases, leptomeningeal metastases, malignant ascites, pleural or pericardial effusions, ascites, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, ill-defined abdominal masses, skin lesions, etc.

Response evaluation:

Recording of Target and New Measureable Lesion Measurements

The longest diameters of non-nodal target and new non-nodal measurable lesions, and short axes of nodal target and new nodal measurable lesions will be recorded. Together they determine the TMTB at follow-up.

Definition of Measurable New Lesions

In order to be selected as new measurable lesions (≤ 2 lesions per organ, ≤ 5 lesions total, per timepoint), new lesions must meet criteria as defined for baseline target lesion selection and meet the same minimum size requirements of 10 mm in long diameter and minimum 15 mm in short axis for new measurable lymph nodes. New measurable lesions shall be prioritized according to size, and the largest lesions shall be selected as new measured lesions.

Non-Target Lesion Assessment

The RECIST 1.1 definitions for the assessment of non-target lesions apply. The response of non-target lesions primarily contributes to the overall response assessments of irCR and irNon-CR/Non-PD (irNN). Non-target lesions do not affect irPR and irSD assessments. Only a massive and unequivocal worsening of non-target lesions alone, even without progress in the TMTB is indicative of irPD.

All new lesions not selected as new measurable lesions are considered new non-measurable lesions and are followed qualitatively. Only a massive and unequivocal progression of new non-measurable lesions leads to an overall assessment of irPD for the timepoint. Persisting new non-measurable lesions prevent irCR.

irRECIST Overall Assessments

irCR: complete disappearance of all measurable and non-measurable lesions. Lymph nodes must decrease to < 10 mm in short axis. Confirmation of response is not mandatory.

irPR: decrease of ≥ 30% in TMTB relative to baseline, non-target lesions are irNN, and no unequivocal progression of new non-measurable lesions.

irSD: failure to meet criteria for irCR or irPR in the absence of irPD.

irNN: no target disease was identified at baseline and at follow-up the subject fails to meet criteria for irCR or irPD.

irPD: minimum 20% increase and minimum 5 mm absolute increase in TMTB compared to nadir, or irPD for non-target or new non-measurable lesions. Confirmation of progression is recommended minimum 4 weeks after the first irPD assessment.

irNE: used in exceptional cases where insufficient data exists.

17.5 Investigator Signature Page

Protocol Title: A Phase I/II, Multicenter, Open-label, Clinical Trial of Intratumoral/Intralesional Administration of RGT100 in Subjects with Advanced or Recurrent Tumors

Protocol Number: RGT100-001

Confidentiality and cGCP Compliance Statement

I, the undersigned, have reviewed this protocol, including appendices, and I will conduct the study as described in compliance with this protocol, GCP, and relevant ICH guidelines. Further I will assure that the protocol is distributed to all involved site personnel as required and that appropriate training is provided.

Once the protocol has been approved by the Ethics Committee, I will not modify this protocol without obtaining prior approval of Rigontec GmbH and the REC. I will submit the protocol amendments and/or any informed consent form (ICF) modifications to Rigontec GmbH and the REC, and approval will be obtained before any amendments are implemented.

I understand that all information obtained during the conduct of the study with regard to the subjects' state of health will be regarded as confidential. No subjects' names will be disclosed. All subjects will be identified by assigned numbers on all eCRFs, laboratory samples, or source documents forwarded to the Sponsor. Clinical information may be reviewed by the Sponsor or its agents or regulatory agencies. Agreement must be obtained from the subject before disclosure of subject information to a third party.

Information developed in this clinical study may be disclosed by Rigontec GmbH, to other clinical Investigators, regulatory agencies, or other health authority or government agencies as required.

_____ _____

Investigator Signature Date

_____

Printed Name

_____

Institution