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NCT Number:	NCT03593226
Sponsor Study Number:	AGI-134.FIM.101
Official Study Title:	A Phase I/IIa, Multicentre, Two-Parts, Open-Label Study Designed to Evaluate the Safety and Tolerability of Escalating Doses of AGI-134 given in Unresectable/Metastatic Solid Tumours
Document Version and Date:	Clinical Study Protocol, Version 6.1, 31 May 2021

CLINICAL STUDY PROTOCOL

A PHASE I/IIA, MULTICENTRE, TWO-PARTS, OPEN-LABEL STUDY DESIGNED TO EVALUATE THE SAFETY AND TOLERABILITY OF ESCALATING DOSES OF AGI-134 GIVEN IN UNRESECTABLE/METASTATIC SOLID TUMOURS

Sponsor:	Agalimmune, Ltd. [redacted]
Investigational Medicinal product:	AGI-134
Protocol Number:	AGI-134.FIM.101
Study Phase:	I/IIa
Sponsor Contact:	[redacted]
Study Pharmacovigilance:	[redacted]
Protocol Version and Date:	Version 6.1, 31 May 2021

Protocol Signature Page

Protocol Title	**A PHASE I/IIA, MULTICENTRE, TWO PARTS, OPEN-LABEL STUDY DESIGNED TO EVALUATE THE SAFETY AND TOLERABILITY OF ESCALATING DOSES OF AGI-134 IN UNRESECTABLE/METASTATIC SOLID TUMOURS**
Protocol Identification	AGI-134.FIM.101
Study Phase	I/IIa
Sponsor	Agalimmune Ltd.

Sponsor Representatives

We, the undersigned, have read this protocol and agree that it contains all necessary information required to conduct the study and that the protocol is in compliance with International Conference on Harmonisation (ICH) and Good Clinical Practice (GCP) guidelines and applicable local regulations.

[redacted] [redacted]

Chief Development Officer Date

[redacted] [redacted]

Chief Medical Officer Date

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GLOSSARY

ADCC	Antibody-dependent cell-mediated cytotoxicity
ADL	Activities of Daily Living
AE	Adverse Event
ALT(SGPT)	Alanine aminotransferase /Serum Glutamic Pyruvic Transaminase
APC	Antigen Presenting Cells
aPTT	Activated Partial Thromboplastin Time
AST(SGOT)	Aspartate Aminotransferase/Serum Glutamic Oxaloacetic Transaminase
CBC	Complete Blood Count
CDC	Complement Dependent Cytotoxicity
CFR	Code of Federal Regulations
CI	Confidence Interval
C_{max}	Maximum plasma concentration
CNS	Central Nervous System
CPI	Checkpoint Inhibitors
CR	Complete Response
CRA	Clinical Research Associate
CrCl	Creatinine Clearance
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Anti-Cytotoxic T-Lymphocyte-Associated protein 4
DCR	Disease Control Rate
DLT	Dose-Limiting Toxicity
dMMR	deficient Mismatch Repair
DNA	Deoxyribonucleic Acid
DOR	Duration of Response
ECG	Electrocardiogram
ECI	Events of Clinical Interest
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FACS	Fluorescence-Activated Cell Sorting
FFPE	Formalin-Fixed Paraffin-Embedded

FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HLT	High Level Term
HNSCC	Head and Neck Squamous Cell Carcinoma
HNSTD	Highest Non-Severely Toxic Dose
HPV	Human Papillomavirus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
INR	International Normalised Ratio (for blood coagulation tests)
IRB	Institutional Review Board
IT	Intratumourally
ITT	Intention-To-Treat
IV	Intravenous
LPLV	Last Patient Last Visit
mAb	Monoclonal Antibody
MedDRA	Medical Dictionary for Regulatory Activities
MHCII	Major Histocompatibility Complex Class II
MM	Medical Monitor
MoA	Mechanism of Action
MRI	Magnetic Resonance Imaging
MSI	Microsatellite Instability
MTD	Maximum Tolerated Dose

NCI	National Cancer Institute
NOAEL	No Observed Adverse Effect Level
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
NSCLC	Non-Small Cell Lung Cancer
ORR	Overall Response Rate
OS	Overall survival
OTC	Over-the-counter
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffered Saline
PD	Progressive Disease
PE	Physical Examination
PET	Positron Emission Tomography
PFS	Progression-Free Survival
PhV	Pharmacovigilance
PI	Principal Investigator
PK	Pharmacokinetic
PP	Per-Protocol
PR	Partial Response
PT	Prothrombin Time
Q2W	Administered every 2 weeks
Q3W	Administered every 3 weeks
RECIST	Response Evaluation Criteria in Solid Tumours
RNA	Ribonucleic acid
RP2D	Recommended Part 2 dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Safety Committee
SD	Stable Disease
SoA	Schedule of Assessments
SOC	System Organ Class
SOPs	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
T1DM	Type 1 Diabetes Mellitus
TCR	T Cell Receptor
TEAE	Treatment-Emergent Adverse Event

TIL	Tumour Infiltrating Lymphocyte
TMB	Tumour Mutation Burden
TMDD	Target-Mediated Drug Disposition
TSH	Thyroid Stimulation Hormone
ULN	Upper Limit of Normal
WBC	White Blood Cell
WES	Whole Exome Sequencing
WHO	World Health Organisation
βhCG	β-human Chorionic Gonadotropin

PROTOCOL SYNOPSIS

Study Title	A Phase I/IIa, Multicentre, Two Parts, Open-Label Study Designed to Evaluate the Safety and Tolerability of Escalating Doses of AGI-134 in Unresectable/Metastatic Solid Tumours
Protocol No.	AGI-134.FIM.101
Clinical Sites	Approximately 15 Sites in UK, Spain and Israel. Additional sites and countries may be added.
Study Phase	I/IIa
Therapeutic Indication	Part 1: AGI-134 in an accelerated escalation - Subjects with superficial, palpable, unresectable/metastatic solid tumours. Part 2: AGI-134 at the Recommended Part 2 Dose (RP2D) - Subjects with selected unresectable/metastatic solid tumours, either superficial or deep lesions.
Scientific Rationale	Humans, apes and Old-World monkeys do not express the α-1,3-galactosyltransferase (α1,3GT) enzyme, which catalyses the synthesis of alpha-Gal epitopes presented on cell surface glycolipids and glycoproteins in other mammals. Humans therefore recognize alpha-Gal epitopes as foreign and, through constant antigenic stimulation by commensal gut bacteria expressing alpha-Gal-like epitopes, produce anti-α-Gal antibodies (termed anti-Gal) in titres as high as 1% of total circulating immunoglobulins. AGI-134 is a synthetic alpha-Gal glycolipid designed to label cells with alpha-Gal, and, by intratumoural administration, to target anti-Gal antibodies to cancer cells and trigger a systemic anti-tumour response. AGI-134 is injected into the tumour where it is incorporated into cancer cell membranes and binds anti-Gal antibodies. Anti-Gal antibodies subsequently activate the complement cascade, trigger complement-dependant cytotoxicity (CDC) and initiate antibody-dependent cellular cytotoxicity (ADCC). This results in the lysis of cells, release of tumour antigens, phagocytosis by antigen presenting cells (APC) and generation of a pro-inflammatory tumour microenvironment. APCs with increased uptake of tumour antigens induce a follow-on systemic immune response by the activation and clonal expansion of T cells (CD8+) to the patient's own tumour cells.

Scientific Rationale	This Phase I/IIa study is designed to assess the safety, tolerability and pharmacokinetics (PK) of AGI-134 and to gain preliminary insight into the clinical activity and variations in measurable tumour biomarkers. According to the proposed mechanism of action (MoA), AGI-134 may be effective in several tumour types.
Study Objectives	The primary objective of the study is to assess the safety and tolerability of AGI-134 injected intratumourally (IT) and to determine the maximum tolerated dose (MTD) and the recommended Part 2 dose (RP2D). Other objectives of this study are to characterise the PK profile of AGI-134 and support the proposed MoA of AGI-134. Efficacy will be assessed as well by clinical and pharmacodynamic parameters.
Study Design	This is a Phase I/IIa, multicentre, open-label study, in subjects with unresectable/metastatic solid tumours. This study comprises of two parts as follow: **<u>Part 1 - Accelerated Dose Escalation of AGI-134</u>** This accelerated dose escalation part is designed to assess the safety and tolerability of escalating doses of AGI-134, as well as determine the MTD and RP2D. A single subject will be dosed per dose level, beginning with a 25 mg dose level and increasing by 100% to 50 mg, 100 mg and up to a maximal dose level of 200 mg. While the concentration of the injected investigational product will be kept constant at 25 mg/mL, the total dose will increase by increasing the total volume injected (1 mL for the 25 mg dose, 2 mL for the 50 mg dose, 4 mL for the 100 mg dose and 8 mL for the 200 mg dose). The calculation of volume for injection to a specific lesion will be based on the longest dimension measured for the superficial and/or palpable lesions selected for injection and according to the guidelines presented in the *Administration of AGI-134 and Tumour Biopsy Procedures in Study AGI-134.FIM.101* document. During the first cycle of treatment for each dose, a subject that experiences a dose-limiting toxicity (DLT) or an adverse event (AE) of moderate or high severity, assessed as related to AGI-134 and as clinically significant, will trigger an expansion of the dose level group to a total of three subjects, and the study will continue in a conventional "3+3" design. If the AE is a DLT, an expansion of the dose level to a total of 6 subjects prior to escalation to the next dose level will be triggered and will be performed in a stepwise manner, dosing an additional two subjects in the first dose expansion step, followed by an additional three subjects that would be dosed only if no second DLT occurs.

Study Design	Moderate injection-site reactions, as well as moderate drug administration-related fever, nausea and vomiting, are expected events with any injectable drug; hence these will not be considered as a trigger for conversion to a "3+3" design. If the cohort was expanded due to a clinically significant related event that is not a DLT, and the expanded dose level group, i.e., 3 subjects, showed no DLT events, then the study will continue to the next dose level and enrol 3 subjects. If 1 out of 3 subjects experienced a DLT in the expanded cohort, a group of 3 additional subjects will be treated at the same dose level. If no more DLTs are reported at that dose, i.e., only 1 out of a total of 6 subjects has developed a DLT, the dose escalation will continue to the next dose level that will include 3 subjects. At any given dose, if 2 or more out of 6 subjects experience a DLT, that dose level will be considered as the DLT dose. In such a case, 3 more subjects will be treated at the previous dose level, if there are less than 6 subjects already treated at that dose. MTD is defined as the highest dose level in which 6 subjects have been treated with less than 2 instances of DLT. When each dose level group completes the first cycle of treatment, (each cycle is 3 weeks long), the safety and tolerability will be assessed by a Safety Committee (SC) to decide whether to escalate the dose or to expand the dose level group. When the MTD is reached or the 200 mg dose (the highest dose proposed to be tested) is found to be safe, whichever comes first, it will be defined as the Recommended Part 2 Dose (RP2D) and the study will continue to Part 2. After completion of the first treatment cycle, subjects in Part 1 of the study will continue receiving intratumoural injections at the dose determined at Screening for 3 additional treatment cycles. No intra-subject escalation will be allowed. The treatment regimen is one dose of AGI-134 per cycle; each cycle is 3 weeks long. The duration of the cycle may be extended if needed to complete additional safety evaluations. Dosing will be given for up to 4 cycles.

Study Design	**<u>Part 2 - AGI-134 at the RP2D</u>** This part is designed to assess the safety, tolerability and biological activity of AGI-134 at the RP2D in subjects with either deep or superficial unresectable/metastatic solid tumours. Subjects with selected unresectable/metastatic solid tumours, either superficial or deep lesions[1], will be enrolled and receive intratumoural injections of AGI-134 according to the injected tumour dimensions, and for a maximum of the RP2D at each dosing, every three weeks for up to 4 cycles.
Study Assessments	This is a two-part study. Each part will comprise of a screening period, a Core study, and a Long-Term Follow-Up period. Details of assessments to be done at each visit are provided within the protocol and the Schedule of Assessments (SoA) (ref to APPENDIX A). **<u>Visit Requirements</u>** **Screening Visit** A window of 28 days is allowed for the Screening period procedures, from the time of Informed Consent Form (ICF) signature until Baseline visit, with the exception of all safety laboratory assessments (CBC, biochemistry, coagulation test), which should be completed within 10 days prior to treatment initiation. Eligibility information will be sent to the Sponsor for review and approval prior to subject's entry into the study. Subjects who fail the Screening procedures may be rescreened after consultation with the Sponsor. Rescreening should include procedures listed in Section 5.1, including re-consent signature. **Baseline Visit** The Baseline visit should occur up to 28 days from the Screening visit. Only subjects who fully comply with the Inclusion/Exclusion Criteria for the study will be eligible for participation and will undergo Baseline visit activities. Treatment initiation (Day 1 visit activities) may be done on the same day of the Baseline, but Baseline procedures should be completed prior to investigational product administration.

[1] Deep lesions are defined as non-palpable lesions, including but not limited to subcutaneous lymph nodes or visceral lesions, injectable with ultrasound or CT guidance. The deep lesions should not be encasing or abutting major vascular structure, e.g., peri-aortic or peri-carotid lesions, or be in a location that is considered high risk for adverse events, e.g., pulmonary parenchyma lesions.

Study Assessments	**Core Study (54 Weeks):** Core study includes Cycles 1-4 visits, Weeks 18-45 visits and Termination Visit. • **Cycle 1, 2, 3 and 4 Visits** This period will begin after completion of the Screening and Baseline visits. On Day 1 of each cycle, AGI-134 will be administered. • **Week 18, 27, 36 & 45 Visits** Subjects will perform the visit at the site every 9 weeks for up to 45 weeks or until progression, whichever comes first. • **Termination/Early Termination Visit (week 54)** A termination visit will take place on Week 54. In case of early termination, the reason should be captured within the electronic Case Report Form (eCRF), and a termination visit must be performed. **Long-Term Follow-Up** All subjects will be contacted by phone every 9 weeks after Termination/Early Termination for 5 years to follow-up on duration of response and survival status. **Safety Analysis and Safety Committee:** The Safety Committee will be composed by the Study Principal Investigator (PI) together with the Sponsor's Medical Monitor (MM) and Pharmacovigilance associate (PhV) or designee, which will be responsible for the review of all the safety data at pre-defined time points. AEs and safety assessments will also be monitored throughout the study on an ongoing basis.

Study Assessments	Safety interim analyses will be conducted as follows: **Part 1:** a) At each dose level after the first subject has completed the first treatment cycle b) After 3 subjects have completed the first treatment cycle if the dose level was expanded c) After 6 subjects have completed the first treatment cycle if the dose level was expanded due to DLT d) Following the first assessment of 3 subjects (or 6 if the dose level was expanded) at each cohort. Safety data will be evaluated by the Safety Committee, who will also meet every 6 months on a regular basis to review ongoing safety data and until the last patient last visit (LPLV). Sites will be required to contact the Sponsor within 24 hours if a subject present with a moderate or severe AE related to AGI-134, that is assessed as clinically significant by the Investigator, during the DLT assessment period (see Section 3.1.1). **Part 2:** During this part, Safety interim analyses will be conducted a) After 3 subjects with deep injectable lesions (or 6 subjects, if expansion is needed) have completed the first cycle of treatment with AGI-134 (i.e., Cycle 1/Day 1 through to Day 21). b) Every 6 months to review ongoing safety data until last patient last visit (LPLV). During this part, 3 subjects will be evaluated after one cycle of injection into deep lesions. If one or more of the subjects' experiences DLTs, a new assessment will be done after the additional 3 subjects with deep lesions injected are enrolled and treated (total 6 subjects). If no new DLTs are reported, the recruitment will continue as planned. If 2 or more subjects presented a DLT, the SC will assess the risk vs. benefit of the treatment and recommend whether or not the dose of AGI-134 should be reduced and/or the treatment schedule be changed (see Section 8.3). **Ongoing Safety Assessment and Dose-Limiting Toxicity** AEs will be reported from the time of informed consent signature Up to 30 days after the last dose of study drug or until the initiation of new anti-cancer treatment, whichever is sooner. SAEs will be reported until 90 days after the last dose of study drug, or until the initiation of new anti-cancer treatment, whichever is sooner.

Study Assessments	The period considered for DLT assessment is defined for Part 1 and Part 2, as the time from the first dose administration of AGI-134 and up to the end of the first cycle of therapy (e.g., Cycle 1/Day 1 through to Cycle 1/Day 21). After the DLT assessment period, data will be assessed an ongoing manner during the study. All AEs should be captured within the eCRF; AEs reported beyond the DLT period will not be considered for the DLT assessment. **General instructions before study drug administration** The following instructions should be followed before AGI-134 administration: • AGI-134 should only be administered when personnel and therapies are immediately available for the treatment of hypersensitivity reactions, should they occur. This should include having an open IV cannula and fluid therapy readily available. • Hypovolaemia, if present, should be corrected prior to administration of AGI 134 according to the study site standard of care. • For patients who receive routine anti-hypertensive medication, these medications should be withheld in the 24 hours prior to injection of AGI-134. Recommendations for patients who experience systemic reactions related to study drug are detailed in section 5.7.
Study Duration	The duration of core study participation is 54 weeks. A Termination visit will be performed for all subjects on Week 54. Long-term follow-up will continue for all subjects for up to 5 years. The study consists of the following study periods: **Screening Period**: up to 28 days **Core Study**: up to 54 weeks **Long-Term Follow-Up:** Following Termination/Early Termination visit, a follow-up telephone call will be performed to all subjects every 9 weeks up to 5 years in order to assess duration of response and survival.
Planned Sample Size	Part 1: Up to 24 subjects. Part 2: Approximately 35 subjects, including ~10 subjects of a pre-defined melanoma sub-population.

Inclusion Criteria	1. Adult male or female aged 18 years or older. 2. Have a histologically or cytologically confirmed unresectable and/or metastatic solid tumour and who have received or been intolerant to all curative treatment options. 3. Subjects should have at least two lesions that are evaluable[2] (based on RECIST v1.1, as determined by the site study team) and that can be assessed for abscopal effect and from which a biopsy can be taken. Superficial large lesions of more than 25 cm can be considered as two lesions upon discussion with and approval by the Sponsor. 4. For a subject to be eligible the following is required: **Part 1:** ▪ Unresectable superficial/palpable tumour feasible for intratumoural injection from any solid tumour, **and** ▪ A metastatic/secondary lesion that can be assessed for abscopal effect and from which a biopsy can be taken. **Part 2:** ▪ At least one unresectable lesion feasible for intratumoural injection and biopsy, **and** ▪ A metastatic/secondary lesion that can be assessed for abscopal effect and from which a biopsy can be taken. The lesions should be from one of the following selected metastatic tumours: ▪ The lesions should be from one of the following selected metastatic tumours: • Skin melanoma patients with progression on up to two lines immunotherapy, whether or not patient was previously treated with chemotherapy or targeted therapy. • Patients with superficial (either visible or palpable) lesions that can be injected and biopsied from the following indications: a. Skin melanoma treated with three previous lines of immunotherapy, upon discussion and approval by the Sponsor. b. Any squamous cell carcinoma (SCC) c. Mucosal melanoma

[2] Tumour lesions situated in a previously irradiated area are considered evaluable if progression has been demonstrated in such lesions.

	d. Other Skin cancers, such as: Basocellular carcinoma, Merkel cell carcinoma e. Any other superficial/palpable tumour, either primary or metastasis that are available for injection and biopsy upon discussion with and approval by the Sponsor. 5. Patients who received prior treatment with CPI should have remained on treatment with one of the immunotherapy treatments for at least 4 months or have stopped treatment earlier than that due to toxicity rather than disease progression. 6. Subjects who are willing to undergo tumour biopsies, according to protocol requirements. 7. Tumour size: o **Part 1**: The total size of the tumour(s) should allow injection of the total volume required at each escalation cohort. The volume can be injected into one lesion or divided into more than one lesion, but the total volume should be given according to the total dose at each escalation part. When volume is divided between several tumours, at least 1 mL should be injected into each tumour. o **Part 2:** Tumour dimensions should allow injection of a minimum of 1 mL. 8. Has ≥ 2 lesions o Has ≥1 injectable lesion which is amenable to injection and biopsy and is evaluable according to RECIST v1.1. o Has another lesion which is amenable for biopsy and is evaluable according to RECIST v1.1. Superficial large lesions of more than 25 cm can be considered as two lesions upon discussion with and approval by the Sponsor. 9. Evaluable Disease according to RECIST v1.1 10. Has an Eastern Cooperative Oncology Group (ECOG) Performance Status score of 0 or 1. 11. Has a life expectancy >3 months

Inclusion Criteria	12. Adequate organ function at Screening as defined below. All laboratory assessments should be performed within 10 days prior to treatment initiation. o Haematology: ▪ White blood cell (WBC) ≥ 2,500/mm^3 ▪ Absolute neutrophil count ≥ 1500 /mm^3 ▪ Platelet count ≥ 100,000/mm^3 ▪ Haemoglobin ≥9 g/dL or ≥5.6 mmol/L ▪ Haematocrit ≥30% o Renal function: ▪ Creatinine ≤1.5x Upper limit of normal (ULN) OR measured or calculated creatinine clearance (glomerular filtration rate [GFR]) can also be used in place of creatinine or CrCl) > 50 mL/min for subject with creatinine levels > 1.5x institutional ULN o Hepatic function: ▪ Total Bilirubin: ≤1.5xULN OR Direct bilirubin ≤ULN for subjects with total bilirubin levels >1.5xULN ▪ AST (SGOT) and ALT (SGPT): ≤2.5xULN OR ≤5xULN for subjects with liver metastases o Coagulation[3]: ▪ International Normalised Ratio (INR) or Prothrombin Time (PT): ≤1.5xULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ▪ Activated Partial Thromboplastin Time (aPTT): ≤1.5xULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

[3] Coagulation test should be within normal limits unless the patients are being treated with anticoagulant in which case the values allowed for injection and biopsy as well as the timing to stop anticoagulants before injection and biopsy will be determined at each site according to institutional guidelines.

Inclusion Criteria	13. Women of childbearing potential and all men must agree to use 2 methods of an adequate contraception: One barrier method (e.g. diaphragm, or condom or sponge, each of which are to be combined with a spermicide) and one hormonal method (e.g. oral, transdermal patch, implanted contraceptives or intrauterine device) prior to study entry and for the duration of study participation through 120 days after the last dose of study treatment. Subjects that are highly unlikely to conceive (e.g. surgically sterile, postmenopausal, or not heterosexually active) are exempt. Confirmation that female subjects are not pregnant must be established by a negative serum β-human chorionic gonadotropin (βhCG) pregnancy test result obtained during Screening. Pregnancy testing is not required for postmenopausal or surgically sterilised women. Non-childbearing potential is defined as (by other than medical reasons): • ≥45 years of age and has not had menses for over 2 years. • <45 years of age and amenorrhoeic for > 2 years without a hysterectomy and oophorectomy and a Follicle Stimulating Hormone (FSH) value in the postmenopausal range upon pre-trial (screening) evaluation. For women, post hysterectomy, bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation and vasectomy for men at least 6 weeks prior to screening. Documented hysterectomy or oophorectomy must be confirmed with medical records of the actual procedure or confirmed by ultrasound. Tubal ligation must be confirmed with medical records of the actual procedure. 14. Subject is able and willing to comply with the requirements of the protocol. 15. Subject is able to voluntarily provide written informed consent.
Exclusion criteria	Subject must be excluded from participating in the study if he/she: 1. Has a disease that is suitable for therapy administered with curative intent. 2. Has any active, acute, or chronic infection(s) that are uncontrolled and/or requiring treatment, such as antibiotics.

Exclusion criteria	3. An active autoimmune disease that has required systemic treatment in the 2 years preceding the study (i.e., with the use of disease-modifying agents, corticosteroids or immunosuppressive drugs). Note: Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment and is allowed. 4. History of or plan for splenectomy or splenic irradiation. 5. History of organ transplant or currently taking active immunosuppressive therapy such as cyclosporine, tacrolimus, etc. 6. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies). 7. Has known active Hepatitis B (e.g., Hepatitis B Surface Antigen [HBsAg] reactive) or Hepatitis C (e.g., Hepatitis C Virus [HCV] RNA [qualitative] is detected). 8. Has known Chronic Hepatitis B or C. 9. History or evidence of cancer associated with immunodeficiency states (e.g. hereditary immune deficiency, organ transplant, or leukaemia). 10. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of study treatment. 11. Is expected to require any other form of antineoplastic therapy while on study. 12. Has positive IgE anti-Gal 13. Subject has a known allergy to alpha-Gal, such as red meat allergy, exposure to lone star tick (Amblyomma americanum), Ixodes ricinus/ holocyclus. 14. Has known allergy or hypersensitivity to any of the test compounds, materials, or contraindication to test product. 15. History or evidence of central nervous system metastases and/or carcinomatous meningitis (unless stable without treatment for at least 6 weeks and not requiring steroids)[4]. 16. Has received other experimental therapies or used an investigational device within 28 days of the first dose of treatment.

[4] Note: Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging using the identical imaging modality for each assessment, either MRI or CT scan, for at least four weeks prior to the first dose of study treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to study treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability

Exclusion criteria	17. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 14 days before first AGI-134 administration or has not recovered from treatment related AE >Grade 1[5]. 18. Has had a prior anti-cancer monoclonal antibody (mAb) treatment within 28 days before first AGI-134 administration or who has not recovered from AE > Grade 1. 19. Is pregnant or breastfeeding or expecting to conceive or father children within the projected duration of the study, starting with the Screening visit through 120 days after the last dose of study treatment. Women with a positive pregnancy test within 72 hours from Baseline. 20. Has unstable angina, new onset angina within the last 3 months, myocardial infarction within the last 6 months, uncontrolled atrial fibrillation, or current congestive heart failure with New York Heart Association Class III or higher. 21. Has a known current additional malignancy that is progressing or requires active treatment. Exceptions are non-melanoma skin lesions, adequately treated basal cell or squamous cell carcinoma that has undergone potentially curative therapy or carcinoma in situ of the cervix. 22. O_2 saturation < 92% (on room air). 23. Has an underlying medical condition that would preclude study participation or other psychological, social, or physical examination (PE) finding or a laboratory abnormality that the PI considers would make the subject a poor study candidate or could interfere with protocol compliance or the interpretation of study results. 24. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the study.

[5] Subjects with ≤ Grade 2 neuropathy or ≤ Grade 2 alopecia are an exception to this criterion and may qualify for the study. If subject underwent major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

Investigational Product Route and Dosage Form	The investigational product in this study is AGI-134. The drug is provided as a 25 mg/mL sterile dispersion in phosphate buffered saline (PBS). In the dose escalation phase, AGI-134 will be administered by intralesional injection at escalating total doses of 25 mg (1 mL), 50 mg (2 mL), 100 mg (4 mL) and up to 200 mg (8 mL) in 3-week cycles until progression, toxicity or 4 cycles completion, whichever comes first. The total volume of the AGI-134 injection may be divided to more than one lesion while maintaining a minimum of 1 mL per injection site. In Part 2 of the study, the treatment schedule will be the same, and the dose of AGI-134 used will be according to the lesion dimensions and up to the RP2D.
Concomitant Medications	All concomitant medications received from 30 days before the Screening visit through 30 days after Termination/ET visit should be recorded. Concomitant medications administered more than 30 days after Termination/ET visit should be recorded if they are part of an SAE's treatment. Medications specifically prohibited in the Exclusion Criteria are not allowed during the study. If there is a clinical indication for any medication specifically prohibited during the study, the study continuation should be discussed with the Sponsor's Medical Monitor. The PI should discuss any questions regarding concomitant medications with the Sponsor's MM. The final decision on any supportive therapy rests with the PI and/or the subject's primary physician. However, the decision to continue the subject's participation in the study requires the mutual agreement between the PI, the Sponsor and the subject. **Acceptable Concomitant Medications:** All treatments that the PI considers necessary for the subject's welfare may be administered at the discretion of the PI in keeping with the acceptable standards of medical care. All concomitant medications, including their indication, will be recorded in the subject's medical file and on the eCRF including all prescription; over the counter (OTC), herbal supplements, and IV medications and fluids. If any change occurs during the study period, documentation of reason for change, drug dosage, frequency, route of administration and date should also be included in the subject's medical file and eCRF.

Concomitant Medications	**Prohibited Concomitant Medications:** Subjects are prohibited from receiving the following medications during the Core study, beginning at Screening visit until Termination visit (week 54) or disease progression, whichever comes first: • Systemic glucocorticoids for any purpose other than to modulate an event of suspected immunologic aetiology or for systemic and local reactions secondary to the treatment. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor. *Note: Inhaled steroids are allowed for management of asthma, and dermatological formulations are allowed to reduce the intensity of injection-site reactions.* • Therapies for the disease under study: o Antineoplastic systemic chemotherapy or biological therapy o Immunotherapy or other therapy not specified in this protocol o Chemotherapy not specified in this protocol o Investigational agents other than AGI-134 o Radiation therapy: localised radiation therapy may be considered on an exceptional case by case basis after consultation with the Sponsor. During the Long-Term Follow-Up period, subjects who have no disease progression should be followed up until disease progression, new cancer treatment or withdraw consent. Subjects may receive other treatments following progression and/or upon investigator's and/or the subject's primary physician decision. For subjects who, in the assessment of the PI, require the use of any of the aforementioned treatments for clinical management, the study continuation should be discussed with the Sponsor's Medical Monitor.

Study Endpoints	Primary Endpoint To assess the safety and tolerability of AGI-134 injected intratumourally, as well as to determine the MTD and the RP2D. Secondary Endpoints • To characterise the PK profile of AGI-134 • To assess the immune response to AGI-134 and to support the MoA • To assess biomarkers that may serve as surrogate markers or predictors of clinical efficacy Exploratory Endpoints • [redacted]

Statistical Analysis	**Analysis Sets:** **Intention-To-Treat (ITT) analysis set:** All enrolled subjects who received at least one dose of investigational product. **Modified Intention-To-Treat (mITT):** All enrolled subjects who received at least one dose of investigational product and have one post Baseline efficacy measurement. **General Statistical Methods:** All measured variables and derived parameters will be listed individually and, if appropriate, tabulated by descriptive statistics. Summary statistics will be provided for all safety, exploratory and Baseline/demographic variables. For categorical variables, frequency tables including percentages will be presented. For continuous variables, descriptive statistics such as number of available observations, mean, median, standard deviation, minimum and maximum will be tabulated. All available data and the tabulation of results will be displayed by initial dose level and with all levels pooled as a whole, if applicable. The data will be analysed as described in the Statistical Analysis Plan. Safety Analysis: Changes in vital signs and routine laboratory data will be presented with descriptive statistics to demonstrate the trend of change. Number of subjects with physical abnormality at each scheduled visit will be tabulated by body system and by dose level. ECG examination results will be displayed in descriptive statistics and by dose level with number of subjects with abnormal findings tabulated for each schedule visit. AE incidence will be summarised descriptively by system organ class (SOC), high level term (HLT) and preferred term (PT) using the latest version of Medical Dictionary for Regulatory Activities (MedDRA; current version 24.0) and by dose level and for the total population. The AEs severity grade, as determined by the latest version of the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE; current version 5.0), and their relationship to study drug will be analysed by SOC, HLT and preferred term by dose level and for all subjects as a group. The action taken, and the outcome will also be analysed accordingly.

Statistical Analysis	By-subject list of toxicity, severity grade will be presented in a time-sequence manner using the latest version of NCI-CTCAE (currently 5.0) with SOC, HLT and preferred term. The number and incidence of subjects experiencing toxicity will be tabulated by worst severity grade in each MedDRA term.

1 INTRODUCTION

1.1 AGI-134

Humans, apes and Old World monkeys do not express the α-1,3-galactosyltransferase (α1,3GT) enzyme, which catalyses the synthesis of alpha-Gal epitopes presented on cell surface glycolipids and glycoproteins in other mammals (U. Galili et al. 1988). Humans therefore recognise alpha-Gal epitopes as foreign and, through constant antigenic stimulation by commensal gut bacteria expressing alpha-Gal-like epitopes, produce anti-alpha-Gal antibodies (termed anti-Gal) in titres as high as 1% of total immunoglobulins throughout life (Galili 1984; Uri Galili et al. 1988; Posekany et al. 2002). When anti-Gal antibodies bind to alpha-Gal-bearing tissue, they activate the complement cascade, trigger complement-dependent cytotoxicity (CDC) and initiate antibody-dependent cellular cytotoxicity (ADCC), resulting in the release of inflammatory mediators and lysis of the tissue.

AGI-134 is designed to label cells with alpha-Gal, and by intratumoural administration, to target anti-Gal antibodies to cancer cells and trigger a systemic antitumour response. Preclinical pharmacology studies have shown that the synthetic glycophospholipid AGI-134 is incorporated into human cancer cell membranes, binds anti-Gal antibodies and induces CDC and ADCC, as well as phagocytosis by antigen presenting cells (APC). Taken together, APCs will have an increased uptake of tumour antigens which will then be cross-presented to effector T cells, to yield a systemic antitumour response. Indeed, animal model studies using galactosyltransferase knockout mice (GT KO) that can generate anti-Gal antibodies with grafted B16-F10 mouse melanoma, showed that treatment with AGI-134 leads to regression of established tumours and protects from secondary tumour development in a dose-dependent and an anti-Gal antibody-dependent manner.

For detailed background information refer to the AGI-134 Investigator's Brochure (IB)

1.1.1 Nonclinical Studies

Nonclinical in-vitro studies of the mechanism of action (MoA) of AGI-134 have shown that AGI-134 incorporates into cell membranes, binds anti-Gal antibodies and induces ADCC and CDC, as well as phagocytosis by APCs. Complement-lysed cells, resulting from AGI-134 incorporation and exposure to serum, were specifically phagocytosed by human APCs and murine CD8α+ dendritic cells, which cross-presented a model antigen to CD8+ T cells.

GT KO mice that do not express the alpha-Gal sugar and can be induced to form anti-Gal antibodies by immunisation with pig kidney homogenate, were used for in-vivo studies of AGI-134. GT KO mice grafted with B16-F10 melanoma showed that intratumoural administration of AGI-134 resulted in primary tumour regression, improved survival and an abscopal effect that protected the mice from development of a distal secondary tumour. In addition, studies showed that a combination of AGI-134 and an anti-PD1 monoclonal antibody had a synergistic benefit in protection from secondary tumour growth.

Nonclinical pharmacokinetic, toxicokinetic and toxicity studies were conducted using animal species that lack alpha-Gal and express anti-Gal antibodies, so that potential effects induced by the interaction of the alpha-Gal portion of AGI-134 and anti-Gal antibodies could be observed.

Relevant animal species include the Cynomolgus monkeys and the transgenic alpha-1,3-galactosyltransferase Knock Out mice.

The study in Cynomolgus monkeys, conducted under good laboratory practice (GLP), included administration of AGI-134 at dose levels of 10 mg/kg (SC) or 50 mg/kg (SC) and 2 mg/kg (IV) or 10 mg/kg (IV) once weekly for four weeks (total of five doses). The highest tested dose (50 mg/kg) is ~ 5-fold higher than the expected clinical dose (as per body surface). All doses were well tolerated. There were no AGI-134-related clinical signs, no AGI-134-related changes in haematology or coagulation and there were no definitive AGI-134-related changes in clinical chemistry or urinalysis parameters.

Based on the above results, the No observed Adverse Effect Level (NOAEL) was considered to be 50 mg/kg (SC) [Day 29 C_{max} of 32,600 ng/mL and 27,600 ng/mL for males and females, respectively and an $AUC_{(0-24)}$ of 444,000 hr*ng/mL and 360,000 hr*ng/mL for males and females, respectively] and 10 mg/kg (IV) [Day 29 C_{max} of 257,000 ng/mL and 253,000 ng/mL for males and females, respectively and an $AUC_{(0-24)}$ of 1,680,000 hr*ng/mL and 1,260,000 hr*ng/mL for males and females, respectively].

For detailed information refer to the AGI-134 IB.

1.1.2 Clinical Trials

This is the first in human clinical study of AGI-134.

1.2 STUDY RATIONALE AND DOSE SELECTION

The selection of the AGI-134 dose is based on the in-vitro and in-vivo efficacy studies in mice and safety studies in Cynomolgus monkeys.

Anti-Gal is a natural anti-carbohydrate antibody and, as such, has a relatively low affinity for alpha-Gal, compared to antibodies raised against peptide antigens. Where anti-peptide antibodies often have nM affinities for their single antigens, anti-Gal has an affinity of ~10 µM for a single alpha-Gal antigen (Wieslander et al. 1990; Wang et al. 1999; Carlson et al. 2007). Hence, to drive the creation of immune complexes and subsequent complement-mediated cytolysis and antibody-dependent cell-mediated cytotoxicity, anti-Gal has to bind to the cells at a high density. To achieve a high cell surface density of anti-Gal, the surface of the tumour cells must be coated with a high density of AGI-134. When the cells are coated with AGI-134 in this way, the spatial arrangement of alpha-Gal on the cell surface drives multivalent antibody binding avidity, which increases the functional affinity of the anti-Gal IgG and IgM molecules from 10 µM to 10 pM. Therefore, high cell surface density of alpha-Gal is required to bind anti-Gal with sufficient avidity to drive downstream effector functions. This is observed and recognised clinically when porcine tissue, which is highly alpha-Gal positive, is rapidly rejected by anti-Gal antibodies in humans (Geller et al. 1992).

The literature, as well as our data, suggest that, to be efficacious, AGI-134 must be injected into the tumour at a concentration that labels as many tumour cells as possible with a sufficient density of alpha-Gal to drive CDC, ADCC and subsequent uptake of tumour debris by APCs. By labelling the tumour cells with a high density of alpha-Gal, multivalent interactions between anti-Gal antibodies and alpha-Gal on the tumour cell surface are more likely, stabilising the binding of anti-Gal antibodies and initiating downstream effector functions.

The concentration of AGI-134 that the tumour cells encounter will dictate the number of AGI-134 molecules that are inserted into the tumour cell membranes. We have demonstrated this in-vitro, where increasing the concentration of AGI-134 increases the molecule incorporation onto the cell membrane, the binding of anti-Gal, as well as increasing functional effect, measured by increased CDC.

For detailed information refer to the AGI-134 IB

In mice, we have demonstrated that increasing the concentration of AGI-134 improves the abscopal efficacy of the alpha-Gal glycolipid in a melanoma model in GT KO mice. Tumours were injected intratumourally with Phosphate Buffered Saline (PBS) (as mock treatment) or AGI-134, and development of the secondary tumour (which represents distant metastases) was monitored. When primary tumour was injected with 100 µL of AGI-134 in concentrations of 1, 5 and 10 mg/mL (total dose: 0.1-1 mg AGI-134), there was increasing efficacy at each concentration (see Section: Non-clinical Pharmacology in the IB). In a side-by-side comparison of using 10 mg/mL and the maximal feasible concentration (solubility-wise) of 25 mg/mL AGI-134, the two formulations had similar potent activity. In addition, AGI-134 in a concentration of 25 mg/mL administered intratumourally resulted in primary tumour regression and had a beneficial effect on survival (see Section: Non-clinical Pharmacology in the IB). In conclusion, 25 mg/mL concentration demonstrated compelling efficacy evidence in-vivo.

Given the above, while the minimal trough concentration to obtain the optimal functionality in-vivo is 10 mg/mL, as tumours in human are expected to vary in their chemical-physical propensities, the maximal feasible concentration will be used (25 mg/mL) for all doses with variable injection volumes. The clinical dose of AGI-134 injected into the tumour will be at a volume that is calculated based on the tumour dimensions, so that as much of the tumour as possible comes into contact with the injected solution.

The anticipated clinical dose range is 25-200 mg per injection (~0.4-3.3 mg/kg in a 60-kg individual). To support the safety of the clinical starting dose and selected dose range, repeated toxicity studies were performed in Cynomolgus monkeys. In order to maximise the opportunity to detect potential toxicities and to account for potential faster clearance in animals relative to humans, a once-weekly treatment schedule was employed. AGI-134 was well tolerated at all tested doses. At the NOAEL doses (50 mg/kg SC and 10 mg/kg IV, highest tested doses), systemic and local exposure was significantly beyond that anticipated clinically. The highest SC dose (50 mg/kg), is 40-fold higher than the anticipated starting dose (based on the human equivalent dose) and 120-fold higher on a weekly human equivalent dose basis. The 50 mg/kg SC is ~ 5-fold higher than the highest expected clinical dose.

The highest IV dose (10 mg/kg) is 8-fold and 24-fold the starting clinical dose on a per dose and weekly dose basis. This dose is expected to cover the highly vascularised nature of some solid tumour types and cover the risk encountered in the worst case of systemic exposure. Based on the 1/6th of the Highest Non-Severely Toxic Dose (HNSTD) approach to estimate a safe starting dose as outlined in ICH S9, the doses of 8.33 mg/kg (1/6th 50 mg/kg dose) and 1.67 mg/kg (1/6th 10 mg/kg) represent multiples of about 20- and 4-fold, respectively, compared with the proposed starting dose of 25 mg per subject. As such, the nonclinical toxicology studies support the selected starting dose and the dose range.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

The primary objective of the study is to assess the safety and tolerability of AGI-134 injected intratumourally, as well as to determine the MTD and the RP2D (in Part 1). Other objectives of this study are to characterise the PK profile of AGI-134 and to support the proposed MoA of AGI-134. Efficacy will be assessed as well by clinical and pharmacodynamic parameters.

2.2 Study Endpoints/ Outcomes

2.2.1 Primary Endpoints

To assess the safety and tolerability of AGI-134 injected, as well as to determine the MTD and the recommended Part 2 dose (RP2D).

2.2.2 Secondary Endpoints

- To characterise the PK profile of AGI-134
- To assess the immune response to AGI-134 and to support the MoA
- To assess biomarkers that may serve as surrogates or predictors of clinical efficacy

2.2.3 Exploratory Endpoints

Exploratory endpoints will include the following efficacy endpoints:

- [redacted]

3 STUDY DESIGN

This is a Phase I/IIa, multicentre, two-parts, open-label study, in subjects with unresectable/ metastatic solid tumours. The study will be comprised of two parts (Figure 1 and Figure 2):

Figure 1: Study Diagram – Part 1

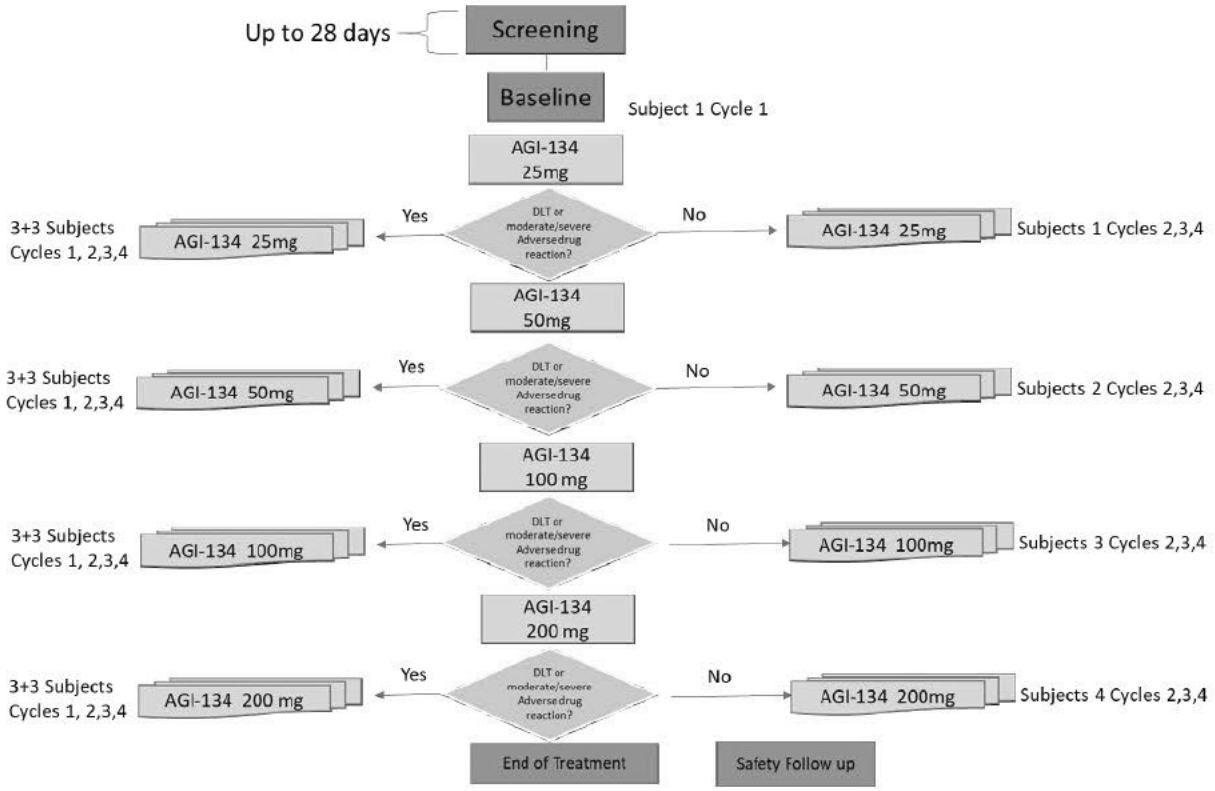
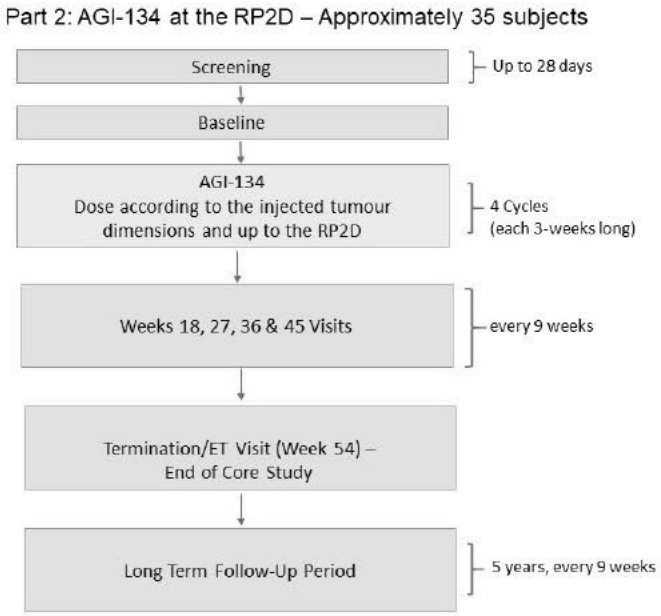


Figure 2: Study Diagram - Part 2



Part 1 - Accelerated Dose Escalation of AGI-134

Part 1 is an accelerated escalation of AGI-134 dose designed to assess the safety and tolerability of AGI-134, as well as to determine the MTD and RP2D.

The total dose will increase incrementally while the concentration will be kept constant at 25 mg/mL, by increasing the total injected volume. A single subject will be administered per dose level beginning with a dose of 25 mg (1 mL) and increasing by 100% to 50 mg (2 mL), to 100 mg (4 mL) and up to a maximal dose of 200 mg (8 mL). The total investigational product volume will be injected in one or more lesions and according to the longest measured dimension of the superficial and/or palpable lesions selected for injection and according to the guidelines presented in the *Administration of AGI-134 and Tumour Biopsy Procedures in Study AGI-134.FIM.101* document. More than one lesion might be injected according to the dose and volume at each escalation level.

At any given dose, if one subject presents with a dose-limiting toxicity (DLT) or an adverse event of moderate or higher severity assessed as related to AGI-134 and as clinically significant during the first cycle of treatment, the group of subjects will be expanded and the study will continue in a conventional "3+3" design. The expansion after an event of moderate severity will be to a total of 3 subjects and the expansion after a DLT will be to a total of 6 subjects, recruited in a stepwise manner of 3+3. Moderate injection-site reactions are expected events with any injectable drug, as are moderate drug administration-related fever, nausea and vomiting. Therefore, these will not be considered as a trigger for conversion to a "3+3" design. If the group of subjects was expanded due to a clinically significant related event that is not a DLT, and this expanded group, i.e. 3 subjects, showed no DLT events, then the study will continue to the next dose level enrolling 3 subjects. If 1 out of 3 subjects presented with a DLT in the expanded cohort, a group of additional 3 subjects will be treated at the same dose level. If no more DLTs

are reported at that dose, i.e. only 1 out of a total of 6 subjects has developed a DLT, the dose escalation will continue to the next level. At any given dose, if 2 or more out of 6 subjects experience a DLT, that dose level will be considered as the DLT dose. In such a case, 3 more subjects will be administered at the previous dose level, if there are less than 6 subjects already treated at that dose. MTD is defined as the highest dose level in which 6 subjects have been administered with and less than 2 instances of DLT.

When the first subject in each dose completes the first cycle of treatment, (each cycle is 3 weeks long), the safety and tolerability will be assessed by a Safety Committee to decide whether to escalate the dose or to expand the group of subjects. When the MTD is reached or the 200 mg dose is found to be safe, whichever comes first, it will be defined as the RP2D and the study will continue to Part 2.

After completion of the first treatment cycle, subjects in Part 1 of the study will continue receiving intratumoural injection at the dose determined at Screening for 3 additional cycles. No intra-subject escalation will be allowed. The treatment regimen is one dose per cycle of AGI-134 every three weeks for up to 4 cycles.

Part 2- AGI-134 at the RP2D

This part is designed to assess the safety, tolerability and biological activity of AGI-134 at the RP2D in subjects with either deep[6] or superficial unresectable/metastatic solid tumours. Subjects with selected unresectable/metastatic solid tumours, either superficial or deep lesions, will be enrolled and receive intratumoural injections of AGI-134 according to the injected tumour dimensions in one or more tumours according to the judgement of the PI and the instructions included in the *Administration of AGI-134 and Tumour Biopsy Procedures in Study AGI-134.FIM.101* document, up to, but not exceeding the RP2D at each dosing, every three weeks for up to 4 cycles.

3.1 Safety Analysis and Safety Committee

The Safety Committee will be composed of the Study PI, the Sponsor's medical monitor (MM) and Pharmacovigilance associate (PhV) or designee. Ad-hoc members may be invited if needed. The committee will be responsible for the review of all the safety data at pre-defined time points to assess the safety of the therapy. AEs and safety assessments will be monitored throughout the study.

[6] Deep lesions are defined as non-palpable lesions, including but not limited to subcutaneous lymph nodes or visceral lesions, injectable with ultrasound or CT guidance. The deep lesions should not be encasing or abutting major vascular structure, e.g., peri-aortic or peri-carotid lesions, or be in a location that is considered high risk for adverse events, e.g., pulmonary parenchyma lesions.

Safety interim analyses will be conducted as follows:

Part 1:

a) At each dose level after the first subject has completed the first treatment cycle.
b) After 3 subjects have completed the first treatment cycle if the dose level was expanded.
c) After 6 subjects have completed the first treatment cycle if the dose level was expanded due to a DLT.
d) Following the first assessment of 3 subjects (or 6 if the dose level was expanded) at each cohort, the SC will meet every 6 months on a regular basis to review ongoing safety data and until the LPLV.

Sites will be required to contact the Sponsor within 24 hours if a subject present with a moderate or severe AE related to AGI-134, that is assessed as clinically significant by the Investigator, during the DLT assessment period (see Section 3.1.1). Mild and moderate injection-site reactions will not be considered as a trigger for conversion to a "3+3" design. Other moderate adverse events that are determined to be not clinically significant by the PI will also not be considered as a trigger for conversion to a "3+3" design (see Section 8.3).

Part 2:

a) After 3 subjects with deep injectable lesions (or 6 subjects, if expansion is needed) have completed the first cycle of treatment with AGI-134 (i.e. Cycle 1/Day 1 through to Day 21).
b) Every 6 months to review ongoing safety data until the last patient last visit (LPLV).

During this part, 3 subjects will be evaluated after one cycle of injection into a deep lesion. If one or more of the subjects' experiences DLTs, a new assessment will be done after additional 3 subjects with deep lesions injected are enrolled and treated (total of 6 subjects). If no new DLTs are reported, the recruitment will continue as planned. If 2 or more subjects presented a DLT, the Safety Committee will assess the risk vs. benefit of the treatment and recommend whether or not the dose of AGI-134 should be reduced and/or the treatment schedule be changed (see Section 8.3: Ongoing Safety Assessment and Dose-Limiting Toxicity).

3.1.1 Ongoing Safety Assessment and Dose-Limiting Toxicity

AEs will be reported from the time of informed consent signature up to 30 days after the last dose of study drug or until the initiation of new anti-cancer treatment, whichever is sooner. SAEs will be reported until 90 days after the last dose of study drug, or until the initiation of new anti-cancer treatment, whichever is sooner. The period considered for DLT assessment for Part 1 is defined as the time from the first dose administration of AGI-134 and up to the end of the first cycle of therapy (i.e. Cycle 1/Day 1 to Cycle 1/Day 21).

The period considered for DLT assessment for Part 2 is defined as the time from the first dose administration of AGI-134 and up to the end of the first cycle of therapy (i.e. Cycle 1/Day 1 to Cycle 1/Day 21) for the first 3 subjects with deep lesions.

After the DLT assessment period, data will be assessed on an ongoing basis during the study. All AEs should be captured within the eCRF, however AEs reported beyond the DLT period will not be considered for the DLT assessment.

During Part 2 of the study, any specific AE that fits the DLT definition and occurs at a frequency of > 30% will stop accrual. In this scenario, the Safety Committee may recommend a lower dose level or dosing schedule change for the continuation of the expanded cohort. The study will be discontinued or terminated in case of an unacceptable risk or a negative change in the risk/benefit assessment. This might include the occurrence of adverse events for which character, severity or frequency is unexpected and overcome the potential benefit of the study drug. In addition, any data deriving from other clinical trials or toxicological studies which negatively influence the risk/benefit assessment might cause discontinuation or termination of the study.

4 STUDY POPULATION

4.1 Inclusion Criteria

1. Adult male or female aged 18 years or older.
2. Have a histologically or cytologically confirmed unresectable and/or metastatic solid tumour and who have received or been intolerant to all curative treatment options and for whom AGI-134 is a reasonable option for treatment.
3. Subjects should have at least two lesions that are evaluable[7] (based on RECIST v1.1, as determined by the site study team) and that can be assessed for abscopal effect and from which a biopsy can be taken. Superficial large lesions of more than 25 cm can be considered as two lesions upon discussion with and approval by the Sponsor.
4. For a subject to be eligible the following is required:

   Part 1:

   - Unresectable superficial/palpable tumour feasible for intratumoural injection from any solid tumour, **and**
   - A metastatic/secondary lesion that can be assessed for abscopal effect and from which a biopsy can be taken.

   Part 2:

   - At least one unresectable lesion feasible for intratumoural injection and biopsy, **and**

[7] Tumour lesions situated in a previously irradiated area are considered evaluable if progression has been demonstrated in such lesions.

- A metastatic/secondary lesion that can be assessed for abscopal effect and from which a biopsy can be taken. The lesions should be from one of the following selected metastatic tumours:
- The lesions should be from one of the following selected metastatic tumours:
  - Skin melanoma patients with progression on up to two lines immunotherapy, whether or not patient was previously treated with chemotherapy or targeted therapy.
  - Patients with superficial (either visible or palpable) lesions that can be injected and biopsied from the following indications:
    a. Skin melanoma treated with three previous lines of immunotherapy, upon discussion and approval by the Sponsor.
    b. Any squamous cell carcinoma (SCC)
    c. Mucosal melanoma
    d. Other Skin cancers, such as: Basocellular carcinoma, Merkel cell carcinoma
    e. Any other superficial/palpable tumour, either primary or metastasis that are available for injection and biopsy upon discussion with and approval by the Sponsor.

5. Patients who received prior treatment with CPI should have remained on treatment with one of the immunotherapy treatments for at least 4 months or have stopped treatment earlier than that due to toxicity rather than disease progression.
6. Subjects who are willing to undergo tumour biopsies, according to protocol requirements.
7. Tumour size:
   - **Part 1:** The total size of the tumour(s) should allow injection of the total volume required at each escalation cohort. The volume can be injected into one lesion or divided into more than one lesion, but the total volume should be given according to the total dose at each escalation part. When volume is divided between several tumours, at least 1 mL should be injected into each tumour.
   - **Part 2:** Tumour dimensions should allow injection of a minimum of 1 mL.
8. Has ≥ 2 lesions
   - Has ≥1 injectable lesion which is amenable to injection and biopsy and is evaluable according to RECIST v1.1.
   - Has another lesion which is amenable for biopsy and is evaluable according to RECIST v1.1. Superficial large lesions of more than 25 cm can be considered as two lesions upon discussion with and approval by the Sponsor.
9. Evaluable Disease according to RECIST v1.1
10. Has an Eastern Cooperative Oncology Group (ECOG) Performance Status score of 0 or 1.
11. Has a life expectancy >3 months

12. Adequate organ function at Screening as defined below. All laboratory assessments should be performed within 10 days prior to treatment initiation.
    - Haematology:
        - White blood cell (WBC) $\geq$ 2,500/mm^3
        - Absolute neutrophil count $\geq$ 1500 /mm^3
        - Platelet count $\geq$ 100,000/mm^3
        - Haemoglobin $\geq$9 g/dL or $\geq$5.6 mmol/L
        - Haematocrit $\geq$30%
    - Renal function:
        - Creatinine $\leq$1.5x Upper limit of normal (ULN) OR measured or calculated creatinine clearance (glomerular filtration rate [GFR]) can also be used in place of creatinine or CrCl) > 50 mL/min for subject with creatinine levels > 1.5x institutional ULN
    - Hepatic function:
        - Total Bilirubin: $\leq$1.5xULN OR Direct bilirubin $\leq$ULN for subjects with total bilirubin levels >1.5xULN
        - AST (SGOT) and ALT (SGPT): $\leq$2.5xULN OR $\leq$5xULN for subjects with liver metastases
    - Coagulation[3]
        - International Normalised Ratio (INR) or Prothrombin Time (PT): $\leq$1.5xULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
        - Activated Partial Thromboplastin Time (aPTT): $\leq$1.5xULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
13. Women of childbearing potential and all men must agree to use 2 methods of an adequate contraception: One barrier method (e.g. diaphragm, or condom or sponge, each of which are to be combined with a spermicide) and one hormonal method (e.g. oral, transdermal patch, implanted contraceptives or intrauterine device) prior to study entry and for the duration of study participation through 120 days after the last dose of study treatment. Subjects that are highly unlikely to conceive (e.g. surgically sterile, postmenopausal, or not heterosexually active) are exempt. Confirmation that female subjects are not pregnant must be established by a negative serum β-human chorionic gonadotropin (βhCG) pregnancy test result obtained during Screening. Pregnancy testing is not required for postmenopausal or surgically sterilised women.

    Non-childbearing potential is defined as (by other than medical reasons):
    - $\geq$45 years of age and has not had menses for over 2 years.

- <45 years of age and amenorrhoeic for > 2 years without a hysterectomy and oophorectomy and a Follicle Stimulating Hormone (FSH) value in the postmenopausal range upon pre-trial (screening) evaluation.

For women, post hysterectomy, bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation and vasectomy for men at least 6 weeks prior to screening. Documented hysterectomy or oophorectomy must be confirmed with medical records of the actual procedure or confirmed by ultrasound. Tubal ligation must be confirmed with medical records of the actual procedure.

14. Subject is able and willing to comply with the requirements of the protocol.
15. Subject is able to voluntarily provide written informed consent.

4.2 Exclusion Criteria

Subject must be excluded from participating the study if he/she:

1. Has a disease that is suitable for therapy administered with curative intent.
2. Has any active, acute, or chronic infection(s) that are uncontrolled and/or requiring treatment, such as antibiotics
3. An active autoimmune disease that has required systemic treatment in the 2 years preceding the study (i.e., with the use of disease-modifying agents, corticosteroids or immunosuppressive drugs). Note: Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment and is allowed.
4. History of or plan for splenectomy or splenic irradiation
5. History of organ transplant or currently taking active immunosuppressive therapy such as cyclosporine, tacrolimus, etc.
6. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
7. Has known active Hepatitis B (e.g., Hepatitis B Surface Antigen [HBsAg] reactive) or Hepatitis C (e.g., Hepatitis C Virus [HCV] RNA [qualitative] is detected).
8. Has known Chronic Hepatitis B or C.
9. History or evidence of cancer associated with immunodeficiency states (e.g. hereditary immune deficiency, organ transplant, or leukaemia)
10. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of study treatment.
11. Is expected to require any other form of antineoplastic therapy while on study.
12. Has positive IgE anti-Gal
13. Subject has a known allergy to alpha-Gal, such as red meat allergy, exposure to lone star tick (Amblyomma americanum), Ixodes ricinus/ holocyclus.
14. Has known allergy or hypersensitivity to any of the test compounds, materials or contraindication to test product

15. History or evidence of central nervous system metastases and/or carcinomatous meningitis (unless stable without treatment for at least 6 weeks and not requiring steroids)[8]
16. Has received other experimental therapies or used an investigational device within 28 days of the first dose of treatment
17. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 14 days before first AGI-134 administration or has not recovered from treatment related AE> Grade 1[9]
18. Has had a prior anti-cancer monoclonal antibody (mAb) within 28 days before first AGI-134 administration or who has not recovered from AE > Grade 1.
19. Is pregnant or breastfeeding or expecting to conceive or father children within the projected duration of the study, starting with the Screening visit through 120 days after the last dose of study treatment. Women with a positive pregnancy test within 72 hours from Baseline.
20. Has unstable angina, new onset angina within the last 3 months, myocardial infarction within the last 6 months, uncontrolled atrial fibrillation, or current congestive heart failure with New York Heart Association Class III or higher.
21. Has a known current additional malignancy that is progressing or requires active treatment. Exceptions are non-melanoma skin lesions, adequately treated basal cell or squamous cell carcinoma that has undergone potentially curative therapy or carcinoma in situ of the cervix.
22. O_2 saturation < 92% (on room air).
23. Has an underlying medical condition that would preclude study participation or other psychological, social or physical examination (PE) finding or a laboratory abnormality that the PI considers would make the subject a poor study candidate or could interfere with protocol compliance or the interpretation of study results.
24. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the study.

4.3 Subject Identification

Each consented subject will receive a unique subject number that will be used to identify the subject for all procedures that occur from Screening throughout the end of study or until the subject terminates from the study. Each subject will be assigned only one subject number. Subject numbers must not be re-used for different subjects.

Any subject who is rescreened will be assigned with a new subject number. Both numbers will be documented in the eCRF for traceability purposes.

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[8] Note: Subjects with previously treated brain metastases may participate, provided they are stable (without evidence of progression by imaging using the identical imaging modality for each assessment, either MRI or CT scan, for at least four weeks prior to the first dose of study treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to study treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability

[9] Subjects with ≤ Grade 2 neuropathy or ≤ Grade 2 alopecia are an exception to this criterion and may qualify for the study. If subject underwent major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

Specific details on the Screening visit requirements (Screening/rescreening) are provided in Section 5.1.

4.4 Screening Failure

Subjects who fail to meet the inclusion criteria at any stage during the Screening period are defined as screening failures. All screening failures will be documented on the appropriate log which will be kept in the Investigator's Site File. Subjects who failed the screening procedures may be rescreened after consultation with the Sponsor. Rescreening procedures are detailed in Section 5.1.

4.5 Removal, Replacement or Early Withdrawal of Subjects from Therapy or Assessment

Subjects are free to discontinue the investigational product and/or their participation in the study at any time and without prejudice to further treatment. The PI must withdraw any subject from the study if that subject requests to be withdrawn, or if it is determined that continuing in the study would result in a significant safety risk to the subject. Subjects that discontinue the investigational product will continue to participate in the Long-Term Follow-up of the study for 5 years, unless they withdraw their consent to continue their participation in the study as well.

Subjects withdrawn from the study prior to the Baseline visit, prior to first injection of AGI-134 or before completion of the DLT assessment period (see Section 3.1.1) will be replaced by the PI to achieve the appropriate number of subjects, regardless of the reason for withdrawal. All data, regardless of DLT period completion or not, will be part of the safety cohort assessment.

If a subject discontinues from the study during the DLT assessment period for any reason other than safety, a replacement subject may be enrolled if deemed appropriate by the PI and Sponsor in order to reach the target number for DLT assessment. Subjects who discontinued the study during the DLT period because of progression will not be considered for DLT assessment; however, they will be counted as having progressed for the efficacy analysis. The replacement subject will be assigned with a unique subject number.

The subject's use of investigational product may be discontinued for any of the following reasons:

- Investigational product toxicity (adverse drug reaction)
- Death
- Request of regulatory agency
- Sponsor’s request
- PI’s request: Investigator decides that withdrawal from the study is in the best interest of the subject
- Withdrawal of consent by the subject
- Disease Progression
- Female subject who becomes pregnant
- Lost to follow-up

- Subject is non-compliant with study procedures/study protocol
- Other

4.6 Handling Withdrawals

If a subject is withdrawn from the study, every effort should be made to determine the reason. This information will be recorded on the subject's eCRF. All subjects who withdraw from the study prematurely, regardless of cause, should undergo all Early Termination study visit procedures (see Section 5.4), followed by a Long-Term Follow-Up.

If withdrawal is caused by an AE that the PI considers as probably related to the investigational product, the AE will be reported to the institutional review board/independent ethics committee (IRB/IEC) as per local national and site guidelines.

All AEs will be followed-up with appropriate medical management until the outcome is determined or stabilised, according to the PI's clinical judgement. All follow-up information will be recorded in the subject's eCRF until resolution of the AE.

Any SAE must be reported to the Sponsor or Sponsor's designee by telephone, fax or email within 24 hours of the PI's becoming aware of the event and to the IRB/IEC according to local regulations (for notification of SAE, refer to Section 7.2.5).

4.7 Termination of Study

The Sponsor reserves the right to discontinue the study at any time at the participating sites for any reason.

Regulatory Authorities also have the right to terminate the study for any reason.

5 STUDY PROCEDURES AND ASSESSMENTS

The Schedule of Assessments (SoA) for this study is shown in APPENDIX A. No protocol-related procedures should be performed before the subject provides a written informed consent. Study-related events and activities (including specific instructions, procedures, concomitant medications, dispensing of study medication, and descriptions of AEs) should be recorded in the appropriate source documents and eCRF.

5.1 Screening Period (Day -28 To Day 0)

At the Screening visit, the purpose and procedures of the study will be fully explained to the subjects. Those wishing to participate in the study will sign a written Informed Consent Form (ICF) prior to initiating any evaluations or study-related procedures. After signing ICF, male and female subjects aged ≥18 years will be screened for study eligibility by assessment of Inclusion and Exclusion criteria. All the assessments should be completed during the Screening period and up to the Baseline visit-Day 0.

A window of up to 28 days is allowed for the Screening period procedures, from the time of ICF signature until Day 0 - Baseline visit (before dosing), with the exception of all safety laboratory assessments (Complete Blood Count (CBC), Biochemistry, coagulation test[10]), which should be completed within 10 days prior to first AGI-134 administration. Eligibility information will be sent to the Sponsor for review and approval prior to subject's entry into the study.

Screening Procedures:

- ICF signature
- Inclusion/Exclusion Criteria review
- Collection of demographic data
- Collection of medical history
- Collection of disease history (including genetic information, dMMR/MSI/TMB)
- Collection of disease treatment history
- Review of prior (up to one year) and concomitant medications
- AE recording
- 12-lead electrocardiogram (ECG) local reading
- Full physical examination
- Vital signs (blood pressures, pulse rate, O_2 saturation, oral temperature in °C, respiratory rate)
- Weight
- Height
- Urinalysis
- ECOG performance status
- Blood will be drawn for:
    - Serum pregnancy test
    - FSH for women who reported menopause for less than two years
    - Disease markers - according to each subject's disease
    - HPV status for relevant indications (HPV status assessment should be performed at local lab in case no available status from the time of diagnosis)
    - PT/INR and Activated Partial Thromboplastin Time (aPTT)[11]
    - CBC with differential
    - Biochemistry panel
    - T3, free T4 and thyroid stimulating hormone (TSH)
    - HIV, HBV and HCV serology

[10] For subjects on anticoagulant treatment, coagulation test should be performed within 24 before the injection and biopsy at Baseline. Anticoagulants should be stopped for injection and biopsy according to institutional guidelines.

[11] The coagulation test should be within normal limits unless the subjects are being treated with an anticoagulant in which case the values allowed for injection and biopsy as well as the timing to stop anticoagulants before injection and biopsy will be determined at each site according to institutional guidelines.

- Blood for anti-Gal IgE

- Tumour imaging. A standard of care imaging performed prior to ICF signature, which were done according to the imaging manual guidelines (Please refer to the *Imaging Manual* for more details) and are within the screening window will be allowed for eligibility.
- RECIST v.1.1 assessment

Subjects may be rescreened after consultation with the Sponsor.

Rescreening Procedures:

- New ICF signature
- Inclusion/Exclusion Criteria review
- AE recording
- 12-lead electrocardiogram (ECG) local reading
- Full physical examination
- Vital signs (blood pressures, pulse rate, O_2 saturation, oral temperature in °C, respiratory rate)
- Weight
- Urinalysis
- ECOG performance status
- Blood will be drawn for:
  - Pregnancy test in serum
  - Disease markers will be collected according to each subject's disease
  - PT/INR and Activated Partial Thromboplastin Time (aPTT)[11]
  - CBC with differential
  - Biochemistry panel
- Tumour imaging will be required upon consultation with the Sponsor's Medical Monitor

5.2 Baseline Visit (Day 0)

Baseline visit (Day 0) procedures occur **before** administration of the investigational product. A window of 3 days is allowed for the procedures and assessments of this visit.

Baseline Procedures:

- Inclusion/Exclusion Criteria re-assessment
- Review of change in concomitant medication
- AE recording
- Symptom-Directed physical examination
- Urinalysis
- Pregnancy test in urine
- Blood will be drawn for:
  - PT/INR and aPTT (results should be available before injection)

- CBC with differential
- Biochemistry Panel

- Biopsies can be performed within 7 days before the first dose of AGI-134 or on the same day of treatment **prior** to AGI-134 administration. Please refer to the instructions included in the *Administration of AGI-134 and Tumour Biopsy Procedures in Study AGI-134.FIM.101* document. Two biopsies should be performed:
  - From the tumour lesion to be injected
  - From an un-injected lesion

Note that dMMR/MSI/TMB assessment may be performed on the collected biopsy and blood sample by the central lab if not available within the Subject's medical history.

It is the Investigator's responsibility to instruct the Subject to contact the site for any changes in his/her well-being. In such cases, Investigator must obtain information regarding concomitant medication changes and adverse events.

5.3 Core Study

The duration of the Core Study will be up to 54 weeks.

AGI-134 administration is divided into cycles (each cycle is 3 weeks long). Each cycle may be extended if additional safety assessment is needed.

AGI-134 is administered for up to 4 cycles. For administration and tissue biopsies information and guidance, please refer to the instructions in the *Administration of AGI-134 and Tumour Biopsy Procedures in Study AGI-134.FIM.101* document.

Visits will be named as follows:

- AGI-134 Administration Visits:
  Visit Cycle 1-Day 1, Visit Cycle 2-Day 1, Visit Cycle 3-Day 1, Visit Cycle 4-Day1
- Other Visits:
  Visit Cycle 1-Day 3, Visit Cycle 1-Day 8, Visit Cycle 1-Day 15, Visit Week 18, Visit Week 27, Visit Week 36, Visit Week 45, Week 54 (Termination)

5.3.1 Cycle 1

This visit will begin after completion of the Baseline visit. Treatment initiation may be done on the same day of Baseline visit, but Baseline procedures should be completed prior to investigational product administration. On this day, AGI-134 will be administered. The following activities will be performed during this visit. Details on the specific days for each assessment are presented in the SoA (APPENDIX A).

Cycle 1 - Day 1 Procedures

- Review of change in concomitant medication
- AE recording

- ECG assessment:
  - A 12-lead ECG Holter recording device will be connected to the subject 1-2 hours prior to AGI-134 administration and record ECG consecutively for at least 24 hours post AGI-134 administration.
  - ECG printouts for safety assessment will be extracted prior to AGI-134 administration (up to 1 hour before dose) and 2 hours post dose (± 30 minutes).
- Symptom-directed physical examination – pre-dose only
- Examination of injection- site will be performed 1 hour (±30 min) pre-AGI-134 administration, 1 hour (±30 min)
- Vital signs will be performed up to 1 hour pre-AGI-134 administration and 0.5, 1, 24, 36, 48 and 72 hrs post AGI-134 administration.
- ECOG performance status
- Photography of superficial injected and non-injected lesions will be performed 1 hour pre-AGI-134 administration (±30 min), 1 hour (±30 min)
- Review general instructions before study drug administration (see section 5.7)
- AGI-134 administration on Day 1
- Blood will be drawn for:
  - CH50 – pre-dose
  - C5a – pre-dose, 2 and 6 hours post AGI-134 administration
  - Flow cytometry – pre-dose and 24 hours post AGI-134 administration
  - Cytokines – pre-dose, 2, 6, 12 and 24 hours post AGI-134 administration
  - Anti-Gal antibodies IgG/IgM and sub classing – pre-dose
  - PBMC isolation + cryopreservation – pre-dose
  - Human leukocyte antigen (HLA) and MHCII haplotyping – pre-dose
  - DNA isolation (MSI/dMMR/TMB, WES) – pre-dose
  - TCR Sequencing – pre-dose
  - RNA isolation (Gene expression/sequencing) – pre-dose
  - PK of AGI-134 and metabolites: up to 1 hour prior to AGI-134 administration and at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48 and 72 hrs post AGI-134 administration. For detailed time window for sample collection refer to the Laboratory Manual.

5.3.2 Cycle 1 - Day 3

- Blood will be drawn for:
  - CBC with differential 48-72 hrs post AGI-134 administration
  - Biochemistry Panel 48-72 hrs post AGI-134 administration
  - Cytokines: 48hrs post AGI-134 administration
  - RNA isolation (Gene expression/sequencing) 48±24hrs post AGI-134 administration
  - PBMC isolation + cryopreservation: 48 ±24 hrs post AGI-134 administration

- Photography of superficial injected lesions prior to tumour tissue biopsy, if applicable
- Examination of injection prior to tumour tissue biopsy, if applicable
- Tumour tissue biopsy will be performed 48 ±24 hrs post AGI-134 administration, from injected lesions only. This biopsy is mandatory for superficial injected lesion and is also requested for deep lesions, unless it is not considered in the best interest for the patient by the PI.

5.3.3 Cycle 1 - Day 8 and Day 15

The following procedures will be performed:

- Review of change in concomitant medication
- AE Recordings
- Symptom-directed physical examination
- Examination of injection site
- Photography of superficial injected and non-injected lesions if applicable
- Vital signs (blood pressures, pulse rate, O_2 saturation, oral temperature in °C, respiratory rate)
- Blood will be drawn for:
  - CBC with differential
  - Biochemistry panel
  - Anti-Gal antibodies IgG/IgM (Cycle 1 - Day 8 only)

5.3.4 Cycles 2, 3 and 4

AGI-134 will be administered on Day 1 of each cycle. The following procedures will be performed during these visits; each assessment should be performed before administration of the investigational product unless otherwise specified. Detailed activities are presented in the SoA (APPENDIX A).

Cycle 2 Procedures

- Review concomitant medications
- AE Recording
- A 12-lead ECG will be recorded prior to AGI-134 administration (up to 1 hour before dose) and 2 hours post dose (± 30 minutes).
- Symptom-directed physical examination – pre-dose only
- Examination of injection site will be performed 1 hour (±30 min) pre-AGI-134 administration and 1 hour (±30 min) post AGI-134 administration
- Vital signs (blood pressures, pulse rate, O_2 saturation, oral temperature in °C, respiratory rate) will be assessed pre-dose and 2 hours post dose
- ECOG performance status
- Photography of superficial injected and non-injected lesions, (if a different lesion is injected the previous injected lesion should be photographed as well): 1 hour (±30 min) pre-AGI-134 administration and 1 hour (±30 min) post AGI-134 administration

- Review general instructions before study drug administration (see section 5.7)
- AGI-134 administration (every effort should be made to inject the same lesion as in previous cycle)
- Urinalysis
- Pregnancy test in urine
- Blood will be drawn for:
  - Disease markers according to subject's disease
  - PT/INR and aPTT (results should be available before injection)
  - CBC with differential
  - Biochemistry panel
  - T3, free T4 and TSH
  - C5a – pre-dose and 2 hours post AGI-134 administration
  - Flow cytometry – pre-dose only
  - PBMCs Isolation + Cryopreservation – pre-dose only
  - Anti-Gal antibodies IgG/IgM and sub classing – pre-dose only
  - PK of AGI-134 and metabolites: up to 1 hour prior to AGI-134 administration and 2 hours post dose

Cycle 3 Procedures

- Review concomitant medications
- AE Recording
- ECG assessment:
  - A 12-lead ECG Holter recording device will be connected to the subject 1-2 hours prior to AGI-134 administration and record ECG consecutively for at least 24 hours post AGI-134 administration.
  - ECG printouts for safety assessment will be extracted prior to AGI-134 administration (up to 1 hour before dose) and 2 hours post dose (± 30 minutes).
- Symptom-directed physical examination – pre-dose only
- Examination of injection site will be performed 1 hour (±30 min) pre-AGI-134 administration, 1 hour (±30 min) and 72 hours (±1 hour) post AGI-134 administration
- Vital signs (blood pressures, pulse rate, O_2 saturation, oral temperature in °C, respiratory rate) will be assessed 1-hour pre-AGI-134 administration and 0.5, 1, 24, 36, 48 and 72 hrs post AGI-134 administration
- ECOG performance status
- Photography of superficial injected and non-injected lesions (if a different lesion is injected, the previous injected lesion should be photographed as well): 1 hour (±30 min) pre-AGI-134 administration and 1 hour (±30 min) and 72 hours (±1 hour) post AGI-134 administration
- Review general instructions before study drug administration (see section 5.7)

- AGI-134 administration (every effort should be made to inject the same lesion as in previous cycle)
- Blood will be drawn for:
  - PT/INR and aPTT (results should be available before injection)
  - CBC with differential
  - Biochemistry panel
  - Blood for anti-Gal IgE – pre-dose (if IgE anti-Gal is detected during assessment at Cycle 3, Cycle 4 dosing with AGI-134 will be cancelled)
  - C5a – pre-dose and 2 and 6 hours post AGI-134 administration
  - Flow cytometry – pre-dose and 24 hours post AGI-134 administration
  - Cytokines – pre-dose and 2, 6, 12 and 24 hours post AGI-134 administration
  - Anti-Gal antibodies IgG/IgM and sub classing – pre-dose only
  - PBMC + cryopreservation – pre-dose only
  - TCR Sequencing – pre-dose only
  - RNA isolation (Gene expression/sequencing) – 24 hours post AGI-134 administration
  - PK of AGI-134 and metabolites: up to 1 hour prior to AGI-134 administration and at 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48 and 72 hrs post AGI-134 administration. For detailed time window for sample collection refer to the Laboratory Manual.
- Biopsies should be performed prior to AGI-134 administration. If not done on the same day of AGI-134 administration, a window of -7 days prior AGI-134 administration is allowed. Two biopsies should be taken:
  - From the tumour lesion to be injected
  - From an un-injected lesion
- Tumour imaging assessment
- RECISTv.1.1 assessment or superficial lesion measurement according to the tumour type will be done on Cycle 3, during Week 9 (before injection of AGI-134 on Cycle 4 - Day 1).

Cycle 4 Procedures

- Review concomitant medications
- AE Recording
- A 12-lead ECG will be recorded prior to AGI-134 administration (up to 1 hour before dose) and 2 hours post dose (± 30 minutes)
- Symptom-directed physical examination – pre-dose only
- Examination of injection site will be performed 1 hour pre-AGI-134 administration and 1 hour (±30 min) post AGI-134 administration
- Vital signs (blood pressures, pulse rate, O_2 saturation, oral temperature in °C, respiratory rate) will be assessed pre-dose and 2 hours post dose.
- ECOG performance status

- Photography of superficial injected and non-injected lesions (if a different lesion is injected, the previous injected lesion should be photographed as well): 1 hour (±30 min) pre-AGI-134 administration and 1 hour (±30 min) post AGI-134 administration
- Review general instructions before study drug administration (see section 5.7)
- AGI-134 administration (every effort should be made to inject the same lesion as in previous cycle).
- Urinalysis
- Pregnancy test in urine
- Blood will be drawn for:
  - Disease markers - according to each subject's disease
  - PT/INR and aPTT (results should be available before injection)
  - CBC with differential
  - Biochemistry panel
  - T3, free T4 and TSH
  - C5a - pre dose and 2 hours post dose
  - PK of AGI-134 and metabolites: up to 1 hour prior to AGI-134 administration and 2 hours post dose

Week 18, 27, 36 and 45 Visits

Visits will be performed at the following weeks: 18, 27, 36 and 45 (i.e. every 9 weeks beginning from Cycle 4).

Week 18, 27, 36 and 45 Visits Procedures:

- Review of change in concomitant medication
- AE Recordings (AEs up to 30 days post last dose of study drug and SAEs up to 90 days post last dose of study drug)
- Examination of injection site
- ECOG performance status
- Photography pictures of all injected superficial tumour, if applicable.
- Tumour Imaging assessment
- RECISTv.1.1 assessment will be done using imaging and/or superficial lesion measurement according to the tumour type.

5.4 Termination or Early Termination Study Visit (Week 54 or ET)

The total study participation period is 54 weeks. Treatment with AGI-134 will be provided for up to 4 cycles (12 weeks). A Termination visit will be performed at Week 54 for all subjects.

In case of Early Termination (ET), all reasons for treatment discontinuation will be documented in the source documents yet only one reason (the most severe) for ET should be recorded in the eCRF. If one of the reasons for early termination is an AE, this should be chosen as the reason. Every effort should be made to follow-up these subjects until resolution or stabilisation of the AE.

At this visit, the following activities will be performed. Exceptions for assessments may be provided in case these assessments were done 1-2 cycles prior to Termination visit and after discussion and approval by the study MM.

Termination / Early Termination Visit Procedures

- Review concomitant medications
- AEs Recording (AEs up to 30 days post last dose of study drug and SAEs up to 90 days post last dose of study drug)
- 12-lead electrocardiogram (ECG) local reading
- Full physical examination
- Examination of injection site
- Vital signs (blood pressures, pulse rate, O_2 saturation, oral temperature in °C, respiratory rate)
- Weight
- ECOG performance status
- Photography of all superficial injected lesions
- Blood will be drawn for:
    - Pregnancy test in serum
    - CBC with differential
    - Biochemistry panel
    - T3, free T4 and TSH
    - Flow cytometry
    - Anti-Gal antibodies IgG/IgM and sub classing
    - PBMC + cryopreservation
    - TCR Sequencing
    - RNA isolation (Gene expression/sequencing)
- In case Early Termination occurred prior to Cycle 3, every effort should be made to perform biopsies on the ET visit. Two biopsies should be taken:
    - From the tumour lesion to be injected
    - From the un-injected lesion

- Tumour Imaging Assessment will be required for termination visit at Week 54 or Early Termination visits, conducted ≥ 9 weeks from last imaging/ RECIST v 1.1 assessment .
- RECIST v 1.1 assessment will be done using imaging and/or superficial lesion measurement according to the tumour type. This assessment will be required for Termination visit at Week 54 or Early termination conducted ≥ 9 weeks from last imaging/ RECIST v 1.1 assessment. Post-Study Anti-Cancer Therapy Status (for early termination)
- Survival and disease status (for early termination)

5.5 Long-Term Follow-Up

All subjects, including early terminated subjects, will be contacted by phone every 9 weeks (±7 days) after Termination/ET visit (Week 54). This Follow-Up will continue for 5 years. During these phone calls, subjects will be asked to provide an update about disease status and post-study anti-cancer therapy received. During the first Long-Term follow-up visit, subjects should be questioned regarding the occurrence of AEs/SAEs and the outcome of any AEs/SAEs that were ongoing at the Termination/Early Termination visit. However, only AEs that occurred up to 30 days after the last dose of study drug and SAEs that occurred up to 90 days after the last dose of study drug will be recorded in the eCRF (see sections 7.1 and 7.2.2 for details). In case of death, every effort should be done in order to capture the date and reason for death. All the data will be collected and entered within the eCRF.

5.6 Unscheduled Visit

An unscheduled visit may be performed at any time during the study at the subject's request or as deemed necessary by the PI. The date and reason for the unscheduled visit will be recorded within the eCRF. AE monitoring and concomitant medication recording will be performed by the PI. Other procedures and evaluations will be completed as deemed necessary by the PI and may include (but are not limited to) laboratory safety tests, vital signs and PE.

5.7 General Instructions before Study Drug Administration

The following instructions should be followed before AGI-134 administration:

- AGI-134 should only be administered when personnel and therapies are immediately available for the treatment of hypersensitivity reactions, should they occur. This should include having an open IV cannula and fluid therapy readily available.
- Hypovolaemia, if present, should be corrected prior to administration of AGI 134 according to the study site standard of care.
- For patients who receive routine anti-hypertensive medication, these medications should be withheld in the 24 hours prior to injection of AGI-134.

<u>In patients who experienced a systemic injection related reaction:</u>

- Premedication with an antihistamine is required prior to all subsequent study drug administrations. Premedication should be given 30-45 minutes prior to study drug

administration if premedication is given orally, or ~15 minutes prior to study drug administration if premedication is given IV.

The time of administration of premedication is to be recorded on site documentation and in the CRF.

The recommended premedication regimen is:

- H1 blocker (e.g diphenhydramine, promethazine, ceterizine)
- **and** H2 blocker (e.g. famotidine).
- Steroids such as dexamethasone should be avoided as premedication; however, short-acting ones may be used to treat injection reactions as needed.

5.8 Safety Assessments

Safety assessments will be based on changes from Baseline visit of clinical signs and symptoms reported by the subject or observed by the PI, including AEs, concomitant medication use, treatment compliance, tolerability (e.g. dropouts due to AEs), vital signs, ECGs, PE and laboratory safety assessments.

5.8.1 Adverse Events

Adverse Events (AE) will be Reported from the time of informed consent signature and up to 30 days after the last dose of study drug or until the initiation of new anti-cancer treatment, whichever is sooner. SAEs will be recorded up to 90 days following the last dose of study drug or until the subject initiates new anti-cancer therapy, whichever is sooner. Any new AE that occurs between scheduled assessment visits should be brought to the attention of the PI and recorded in the Subject's medical file and on the appropriate eCRF page.

AEs will be reported and graded in accordance with the latest National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version (currently version 5.0) and coded by Data Management using the latest version of Medical Dictionary for Regulatory Activities (MedDRA, version 24.0; see Section 7.1 for more details).

5.8.2 Concomitant Medication

Review of prior medication (up to one year) and concomitant medications use will be recorded from Baseline visit through all study visits.

5.8.3 Vital Signs, Height and Weight

- Vital signs will be measured once at Screening and once at Termination/Early Termination visit.
- On Cycle 1 - Day 1 and Cycle 3 - Day 1 will be measured up to 1 hour pre-AGI-134 administration and 0.5, 1, 24, 36, 48 and 72 hrs post dose.
- On Cycle 2 - Day 1 and Cycle 4 - Day 1 will be measured pre-dose and 2 hours post dose.

Vital signs will include blood pressure, pulse, oral temperature, O_2 saturation and respiration rate following a minimum of 5 minutes rest and prior to blood collection as per standard practice at the investigational site. Significant findings noticed after investigational product administration which meet the definition of an AE must be recorded on the AE eCRF module.

Height and weight will be recorded at Screening. Weight will be recorded at Termination/Early Termination visit as well.

5.8.4 Electrocardiogram

Local reading:

12-Lead ECG will be recorded for all subjects in both study parts as follows:

- Screening Visit
- Cycle 1, 2, 3 and 4 at: prior to AGI-134 administration (up to 1 hour before dose) and 2 hours post dose (± 30 minutes). In Cycles 1 and 4, ECG printouts for safety assessment may be taken from the Holter recording device.
- Termination/Early Termination Visit

Central reading:

- Cycle 1 and 3: 12-lead ECG Holter recording device will be connected to the subject 1-2 hours prior to AGI-134 administration and record ECG consecutively for at least 24 hours after AGI-134 administration.

5.8.5 Physical Examination and Symptom-Directed Physical Examination

Complete physical examination (PE) will be conducted at screening and at Termination/ET. PE will include assessment of the following body systems: Head, neck, thyroid, respiratory, cardiovascular, ophthalmologic, gastrointestinal, hepatic, musculoskeletal system, dermatological, lymph nodes, neurological system and, where appropriate, other body systems as indicated in the study schedule.

A limited, symptom-directed PE (Directed PE) should be performed on Day 1 pre-dose of each cycle. During a Directed PE, particular attention should be focused on identifying possibly drug-related AEs. It is important to carefully inspect the lesion's site of injection and site biopsy. Changes in PE since last visit will be assessed and, if assessed as clinically significant, it should be reported within the eCRF as an AE. Information about the PE must be presented in the source documentation at the study site. Significant findings that are present prior to the investigational product administration must be included in the Relevant Medical History/ Current Medical Conditions eCRF page. Significant findings made after investigational product administration, which meet the definition of an AE, must be recorded on the AE eCRF module.

5.8.6 Laboratory Safety Assessment

All clinical laboratory safety assessments, listed below, will be performed by local laboratories at the participating sites at Screening and at different time points during treatment depending on the specific assessment. Pre-dose laboratory procedures may be conducted up to 4 hours prior to dosing.

Laboratory safety sampling will include the parameters listed below. The exact time points for each of the tests are specified in the SoA (APPENDIX A). Safety Biomarker will be assessed as well, as defined in Table 2.

Laboratory tests for haematology, chemistry, urinalysis and others are specified in Table 1.

Table 1: Laboratory Tests

Haematology	Chemistry	Urinalysis	Other
Haematocrit	Albumin	Blood	Follicle Stimulating Hormone (FSH)
Haemoglobin	Alkaline phosphatase	Glucose	Serum or urine pregnancy test
Platelet count	Alanine aminotransferase (ALT)	Protein	Hepatitis B Surface Antigen [HBsAg] reactive
WBC (total and differential)	Aspartate aminotransferase (AST)	Specific gravity	Hepatitis C (e.g., HCV RNA [qualitative]
RBC	Calcium	Microscopic examination	HIV1 and 2
	Chloride		TSH, T3 (free or total) and freeT4
	Creatinine		PT/INR and aPTT
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Total Bilirubin		
	Direct Bilirubin		
	Total protein		
	Blood Urea Nitrogen		
	Lactic dehydrogenase (LDH)		

Table 2: Safety Biomarkers

Test	Scientific Rationale	Frequency of Test	Levels Quantified in Terms of Actionable Items
Anti-Gal IgE	Acute hypersensitivity may be caused by presence of serum anti-Gal IgE antibodies in humans, which are associated with allergies to red meat and induced by bites from the Lone Star Tick, *Amblyomma americanum*.	Screening period (Exclusion Criteria) Blood for anti-Gal IgE on Cycle 3 before injection of AGI-134 (timepoint selected based on isotype switching kinetics)	Positive detection of IgE anti-Gal at Screening will exclude the subject from the study. If IgE anti-Gal is detected during assessment at Cycle 3, dosing with AGI-134 will be discontinued.

Laboratory safety test abnormalities that arise after investigational product administration will be repeated as clinically indicated until the values return to normal or until the aetiology has been determined and the condition considered stable. Abnormal laboratory test results that are considered to be clinically significant by the Site PI will be reported as AEs in the AE eCRF module.

A laboratory abnormality will not be considered an AE unless it fulfils one of the following:

- It is clinically significant according to the PI's judgement
- Intervention is required
- Changes in dose are required (decrease, discontinued, interrupted)
- Other treatment/therapy is required
- Associated with other diagnoses

Laboratory results will be reported to the PI or designee who will review, sign and date abnormal laboratory findings for clinical significance. The PI will note any laboratory test results of clinical concern or values that were outside normal ranges and provide details of the relationship to the investigational product and the action taken. If a change in a laboratory value represents a medical condition, the medical condition will be listed in the AE record. If no correlation is possible, the direction of change (increase or decrease) in addition to the actual value will be recorded.

5.9 ECOG Performance Status

ECOG performance status (see APPENDIX C) will be collected at each visit, including Termination. ECOG performance assessment should be performed prior to drug administration.

5.10 Pharmacodynamic Evaluations

The pharmacodynamic assessments, listed below in Table 3, will be performed by either the local laboratory at the participating sites and/or designated central laboratories according to the Laboratory Manual. The techniques and specific markers for assessments will be defined in the Laboratory Manual.

Table 3: Pharmacodynamic and Biomarkers Assessments

Test	Scientific rationale	Frequency of test
HLA and MHCII Haplotyping	Biomarker for immune response	Cycle 1 (pre-dose)
DNA isolation (MSI/dMMR/TMB/WES) blood	Biomarker for immune response	Cycle 1 (pre-dose)
DNA isolation (MSI/dMMR/TMB/WES) tissue	Biomarker for immune response	Baseline
CH50	Biomarker for immune response	Cycle 1 (pre-dose)
C5a	Biomarker for immune response	Cycle 1 (pre-dose, 2h and 6h post dose), Cycle 2 (pre-dose and 2h post dose), Cycle 3 (pre-dose and 2h and 6h post dose) and Cycle 4 (pre-dose and 2h post dose)
Flow cytometry	Biomarker for immune response	Cycle 1 (pre-dose and 24h post dose), Cycle 2 (pre-dose), Cycle 3 (pre-dose and 24h) and Termination

Test	Scientific rationale	Frequency of test
Cytokines	Biomarker for immune response	Cycle 1 (pre-dose, 2, 6, 12, and 24h), Cycle 3 (pre-dose, 2, 6, 12, 24h post dose) and Termination
PBMCs isolation + cryopreservation	Biomarker for immune response	Cycle 1 (pre-dose and 3 days post injection), Cycle 2 (pre-dose), Cycle 3 (pre-dose) and Termination
Anti-Gal IgG/IgM	Biomarker for immune response	Cycle 1 (pre-dose and Day 8), Cycle 2 (pre-dose), Cycle 3 (pre-dose), Cycle 4 (pre-dose) and Termination
Anti-Gal Sub classing	Biomarker for immune response	Cycle 1 (pre-dose), Cycle 2 (pre-dose), Cycle 3 (pre-dose), Cycle 4 (pre-dose) and Termination
TCR sequencing blood	Biomarker for immune response	Cycle 1 (pre-dose), Cycle 3 (pre-dose) and Termination
TCR sequencing tissue	Biomarker for immune response	Baseline, Cycle 3 (pre-dose) and Termination
IHC/H&E/Multiomyx	Biomarker for immune response	Baseline, Cycle 1 (Day 3), Cycle 3 (pre-dose) and Termination
RNA isolation (Gene expression/sequencing) blood	Biomarker for immune response	Cycle 1 (pre-dose and Day 3), Cycle 3 (24h post-dose) and Termination
RNA isolation (Gene expression/sequencing) tissue	Biomarker for immune response	Baseline, Cycle 1 (Day 3), Cycle 3 and Termination

5.11 Planned Genetic Analysis

Genetic analysis will be performed on blood and on available biopsy samples. Understanding genetic determinants of drug response is an important endeavour during medical research. This research will evaluate whether genetic variation within a clinical study population correlates with response to the treatment(s) under evaluation.

5.12 Efficacy Assessments

Response will be assessed by the PI using RECIST v1.1 (see APPENDIX B). Assessments should be performed by the same evaluator, if possible, to ensure internal consistency across visits. Results must be reviewed by the PI before dosing at the next cycle. Please refer to the *Imaging Manual* for more details.

Subjects will undergo tumour assessments at different timepoints as described below:

- Screening Visit
- Cycle 3 (week 9) and further every 9 weeks (weeks 18, 27, 36, 45) including Termination/Early Termination Visit

If radiologic imaging verifies initial PD, at PI's discretion, tumour assessment can be repeated ≥4 weeks later and no later than the next schedule CT scan in order to confirm PD with the option of continuing treatment as described below.

All measurable and evaluable lesions should be assessed and documented at Screening. Tumour assessments performed as standard of care prior to obtaining informed consent and within 28 days prior to initiation of study treatment do not have to be repeated at Screening.

The same method of assessment and the same technique should be used to characterise each identified and reported lesion at Baseline and during follow-up. Imaging-based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

If a CT scan for tumour assessment is performed in a positron emission tomography (PET)/CT scanner, the CT acquisition must be consistent with the standards for a full contrast diagnostic CT scan.

All measurable and evaluable lesions should be re-assessed at each subsequent tumour evaluation. The same radiographic procedures used to assess disease sites at Screening should be used for subsequent tumour assessments (e.g., the same contrast protocol for CT scans).

5.12.1 Direct Measurement of Superficial Lesions

Imaging-based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. Clinical assessment of lesions will only be considered measurable when they are superficial and ≥10mm dimension as assessed using callipers (e.g. skin nodules). For the case of skin lesions, documentation by colour photography, including a ruler to estimate the size of the lesion, should be used. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

While evaluation of lesions by PE is also of limited reproducibility, it is permitted when lesions are superficial, at least 10 mm size, and can be assessed using callipers. In general, it is preferred if subjects on clinical studies have at least one lesion that is measurable by CT. Other skin or palpable lesions may be measured on PE and be considered target lesions.

For response criteria assessment, documentation of palpable lesion size should be performed by photographing the lesion after circumscribing the lesion with a marker, delineating the long axis of the lesion with fingers, and placing a ruler adjacent to the lesion. This approach should be repeated at subsequent evaluation time points, with reference made to prior photographs to ensure that the same long axis measurements are performed consistently. The long axis diameter in millimetres should be used in the SOD calculation in combination with other target lesion(s).

5.12.2 Planned Biopsy Analysis

Biopsies will be performed at baseline and Cycle 3 from injected lesion as well as from un-injected lesion.

Additional biopsy from superficial injected lesion will be performed on Cycle 1 Day 3 (48-72 hours) post AGI-134 administration. This biopsy is mandatory for superficial injected lesions

and is also requested for deep lesions, unless not considered in the best interest for the patient by the PI.

Procedures, collection, storage and shipping instruction will be provided in the *Administration of AGI-134 and Tumour Biopsy Procedures in Study AGI-134.FIM.101* document.

The reason for a missing biopsy assessment should be documented within the eCRF. All efforts should be done in order to conduct all the biopsy assessments as required in the protocol.

Two core biopsies are required to be taken at each timepoint and from each lesion. All biopsies will be processed as Formalin-Fixed Paraffine-Embedded (FFPE) blocks.

6 INVESTIGATIONAL PRODUCT

6.1 Identity of AGI-134

AGI-134 drug product is provided as 25 mg/mL in PBS, Solution for Injection. AGI-134 is filled and packed in clear Type I glass vials (DIN 2R), 1 mL per vial, with 13 mm serum stoppers and sealed with 13 mm caps.

6.2 Investigational Product Administration and Dosage

Each dose of AGI-134 will be administered intratumourally (IT) according to the longest tumour dimension and as described in the *Administration of AGI-134 and Tumour Biopsy Procedures in Study AGI-134.FIM.101* document.

6.3 Manufacturing of Study Medication

AGI-134 is provided as a solution for injection administered via intratumoural injection. The drug product is a clear liquid essentially free of visible particles. The product is manufactured according to Good Manufacturing Practice (GMP) by [redacted].

6.4 Distribution and Shipment of Study Medication

The investigational products will be packed and shipped in appropriate boxes. If, upon arrival at the clinical investigation site, the investigational product supplies appear to be damaged, the Clinical Research Associate (CRA) should be contacted immediately.

Each shipment of investigational product supplies for the study will be accompanied by a shipment form describing the contents of the shipment, product certificate of analysis, acknowledgement of receipt and other appropriate documentation. The shipment form will assist in maintaining current and accurate inventory records. The study staff will confirm the receipt of clinical supply to the CRA.

All study supplies should arrive at the pharmacy/Investigational site in sufficient quantity and in time to enable dosing as scheduled. The Sponsor or its representative must notify the PI prior to dispatch of drug supplies, with the anticipated date of their arrival.

6.5 Storage, Dispensing and Return of the Investigational Product

Investigational product dispensing and accountability records (how much investigational product was dispensed to each subject) should be kept by the Pharmacy and by the PI or designee. The CRAs must periodically check the investigational product supplied to ensure expiry date and sufficient amount of investigational product and be sure that drug accountability is being performed at each visit and the drug accountability logs are maintained.

The investigational product must be kept in a secure area with a limited-access to the investigational product by designated study personnel.

Only trained personnel under the supervision of either the PI or the local pharmacist are authorised to dispense and administer investigational product to participating subjects.

Further details and instructions will be provided in the Pharmacy Manual.

AGI-134 solution for injection 25 mg/1 mL vials should be stored at refrigerated conditions (2-8°C), protected from light and at vertical, upright position.

6.6 Accountability and Compliance of Investigational Product

Each delivery must be acknowledged by the site's pharmacist (or authorised study team member responsible for the investigational product) by filling in the receipt record form and returning it by fax/email to the Sponsor or designee. Accurate, complete and timely documentation of investigational product distribution will be maintained by the pharmacy and the study staff of the investigational site which may include confirmation of receipts of clinical supply, drug accountability logs and other forms.

The site's pharmacist (or authorised study team member responsible for the investigational product) is responsible for ensuring the supervision of the storage and allocation of these supplies, which will be forwarded to the PI at the appropriate time before administration. The PI will dispense investigational product only to subjects enrolled in the study.

Drug accountability records must be maintained by the site at all times. At the last study visit, all used and unused investigational product will be collected, and drug accountability will be performed by the study staff.

During the study, drug orders, records of investigational product receipts, dispensing records and inventory forms located at the site will be checked and reconciled by the CRA periodically during and at the end of the study.

The subject number, the date, batch number/pack number and quantity of investigational product used by the subject will be checked for correctness and recorded on the appropriate accountability forms. Unused drug supplies will be returned to the Sponsor. At the end of the study, all clinical supplies and the corresponding accountability forms must be returned to the Sponsor, the CRA or designee for reconciliation or destruction. A photocopy of these records must be kept at the clinical investigation site.

6.7 Concomitant Therapy

At the Screening visit, relevant treatments currently taken by the subject will be recorded in the subject's eCRF including treatment's name, indication, dose, total daily dose and start- and stop dates.

Any medications (including prescription, over-the-counter [OTC], herbal supplements and other health store-type products) to be taken during the study must be approved by the PI.

Concomitant medication use will be recorded from Screening through the Termination visit. Treatment provided for the subject's disease, during Long-Term Follow-Up will be captured as part of the duration of response and survival status assessment. The following information should be recorded: Treatment's name (generic, if possible) dose and start- and stop dates.

All concomitant medications received from 30 days before the Screening visit through 30 days after Termination/ET visit should be recorded. Concomitant medications administered more than 30 days after Termination/ET visit should be recorded for SAEs and events of clinical interest (ECIs).

Medications specifically prohibited in the Exclusion Criteria are not allowed during the ongoing study. If there is a clinical indication for any medication specifically prohibited during the study, the study continuation should be discussed with the Sponsor's Medical Monitor. The PI should discuss any questions regarding this with the Sponsor's MM. The final decision on any supportive therapy rests with the PI and/or the subject's primary physician. However, the decision to continue the subject's study therapy requires the agreement of the PI, the Sponsor and the subject.

6.7.1 Allowed Medications

All treatments that the PI considers necessary for a subject's welfare may be administered at the discretion of the PI in keeping with the community standards of medical care. All concomitant medication will be recorded on the eCRF including all prescription, OTC, herbal supplements, and IV medications and fluids. If changes occur during the study period, documentation of drug dosage, frequency, route and date may also be included in the eCRF.

6.7.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following medications during the Core study, beginning at Screening Visit until Termination Visit (week 54) or disease progression, whichever comes first:

- Systemic glucocorticoids for any purpose other than to modulate an event of suspected immunologic aetiology or for systemic and local reactions secondary to the treatment. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor. *Note: Inhaled steroids are allowed for management of asthma, and dermatological formulations are allowed to reduce the intensity of injection-site reactions.*
- Therapy for the disease under study:
    - Antineoplastic systemic chemotherapy or biological therapy
    - Immunotherapy or other therapy not specified in this protocol

- Chemotherapy not specified in this protocol
- Investigational agents other than AGI-134
- Radiation therapy: localised radiation therapy may be considered on an exceptional case by case basis after consultation with the Sponsor.

During the Long-Term Follow-Up period, subjects who have no disease progression should be followed up until disease progression, new cancer treatment or withdraw consent. Subjects may receive other treatments following progression and/or upon investigator's and/or the subject's primary physician decision.

For subjects who, in the assessment of the PI, require the use of any of the aforementioned treatments for clinical management, the study continuation should be discussed with the Sponsor's Medical Monitor.

The Exclusion Criteria describes other medications that are prohibited in this study.

6.7.3 Contraception

AGI-134 effect on foetus in utero was not tested to date.

For this study, male subjects will be of non-reproductive potential, if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Males and non-pregnant, non-breastfeeding females may only be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as;

1) surgically sterilised

2) Women of non-childbearing potential (see definition in section 4.1 Inclusion Criteria)

3) not heterosexually active for the duration of the study when this is in line with the preferred and usual lifestyle of the subject.

The two birth control methods can be a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from Screening visit (or 21 days prior to investigational product administration for oral contraception) and throughout the Core Study up to 120 days after the last dose of study medication.

The following are considered adequate barrier methods of contraception: Diaphragm, condom (by the partner) or sponge; each of which are to be combined with a spermicide or a copper intrauterine device as per local regulations or guidelines. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an oestrogenic and/or a progestational agent (including oral, subcutaneous, intrauterine or intramuscular agents).

Subjects should be informed that taking the study medication may involve unknown risks to the foetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, subjects of childbearing potential must adhere to the contraception requirement (described above) for the duration of the study and 120 days after the last dose of study treatment. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

6.7.3.1 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with investigational product, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly or other disabling or life-threatening complication to the mother or new-born). The Study PI will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the foetus or new-born to the Sponsor. If a male subject impregnates his female partner, the site personnel must be informed immediately, and the pregnancy reported to the Sponsor and followed as described above and in Section 7.2.6 Reporting of Pregnancy and Lactation to the Sponsor.

6.7.3.2 Use in Nursing Women

Since it is unknown whether AGI-134 is excreted in human milk and due to the potential for serious adverse reactions in the nursing infant, subjects who are breastfeeding are not eligible for enrolment.

6.7.4 Dose Modifications

AGI-134 concentration is fixed. In Part 1, doses will be escalated starting with 25 mg up to 200 mg. Each dose can be injected into one lesion or can be divided to several lesions, depending on the lesion size. When the MTD is reached or the 200 mg dose is found to be safe, whichever comes first, the RP2D will have been determined and the study will continue to Part 2. In Part 2, the dose will be deduced according to the tumour dimensions and up to a maximum dose according to the RP2D.

Interruptions from the protocol-specified treatment more than the allowed below, require consultation between the PI and the Sponsor and written documentation of the collaborative decision on subject management.

Dosing interruptions are permitted in case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, subject vacation, and/or holidays). Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor's MM. The reason for interruption should be documented in the subject's study record.

This is a first-in-human study with AGI-134, therefore, no dose modifications are proposed. The dosing of AGI-134 will be explored by an escalation phase followed by the expansion phase. If modifications are needed, they will be explored within this study.

6.7.4.1 Rescue Medications & Supportive Care Guidelines for AGI-134

This is a first-in-human study with AGI-134, therefore, no specific guidelines are pre-defined. AE should be treated according to the PI best clinical judgement.

7 SAFETY PHARMACOVIGILENCE

7.1 Adverse Events

An AE is defined in the International Conference on Harmonisation (ICH) E6 as "any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product."

An abnormal result of diagnostic procedures including abnormal laboratory findings will be considered an AE if it fulfils one or more of the following:

- Results in subject's withdrawal by the PI
- Is associated with an SAE
- Is associated with clinical signs or symptoms
- Is considered by the PI to be of clinical significance

A new condition or the worsening of a pre-existing condition will be considered an AE.

AEs do not include the following:

- Medical/surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion). The condition that leads to the procedure is an AE if the procedure was not planned at Screening visit.
- Overdose of concomitant medication without any signs or symptoms unless the subject is hospitalised for observation.
- Hospitalisation for elective surgery planned prior to study (situation where an untoward medical occurrence has not occurred).
- Progression of the cancer under study, unless it is considered to be drug-related by the PI.

Once the subject has signed the Informed Consent Form (ICF), AEs will be recorded up to 30 days after the last dose of study drug, or until the initiation of new anti-cancer therapy, whichever sooner. SAEs will be recorded up to 90 days following the last dose of study drug or until the subject initiates new anti-cancer therapy, whichever is sooner. All AEs, whether observed by the PI or designee or volunteered by or elicited from the subject, should be recorded individually on an AE eCRF page with the following information: The specific event or condition, whether the event was present pre-treatment initiation or not, the dates and times (using the 24 hour clock, where midnight is 00:00 and noon is 12:00) of occurrence, duration, severity, relationship to study medication or to the study procedures of drug administration or biopsy, action taken with the investigational product, outcome, and whether considered non-serious or serious, drug-related or not.

The relationship of the adverse event to the study medication should be assessed by the PI or designee and recorded accordingly. Causality will be reported as follows:

- Likely related to the study medication
- Likely related to the procedure: i.e. study drug injection and/or biopsy
- Likely related to disease under study
- Not related
- Other, if none of the above is applicable

The severity of the AE will be assessed by the PI in accordance with the definitions below. A Serious AE must fulfil the requirements listed in Section 7.2.

AEs severity (Table 4) will be recorded and graded according to the latest version of the NCI-CTCAE (currently version 5.0) and coded into the database according to the latest version of MedDRA (currently version 24.0).

Table 4: Severity of Adverse Events According to CTCAE (Version 5.0)

Grade	Description
0	No AE or within normal limits
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)
3	Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care ADL
4	Life-threatening consequences; urgent intervention indicated
5	Death related to AE

A semi-colon indicates 'or' within the description of the grade.

A single dash (-) indicates a grade is not available

The following definitions should be used for toxicities/AEs that are not defined in the CTCAE:

- Mild (Grade 1): The AE is noticeable to the subject but does not interfere with routine activity, no medical intervention is required;
- Moderate (Grade 2): The AE interferes with routine activity but responds to symptomatic therapy or rest;
- Severe (Grade 3): The AE significantly limits the subject's ability to perform routine activities despite symptomatic therapy;
- Life-threatening (Grade 4): The subject is at immediate risk of death.

The PI will document his opinion of the relationship of the AE to treatment with investigational product using the criteria outlined in Table 5.

Outcome to Date are classified as follows:

- Recovered: The subject has fully recovered from the AE with no observable residual effects
- Recovered with sequelae: The subject has recovered from the AE with observable residual effects
- Improved: The subject status has improved but is not fully recovered
- Ongoing: AE is not recovered
- Fatal
- Unknown

AEs will be coded by Data Management using the latest version of MedDRA (currently version 24.0) AE dictionary.

All AEs, serious and not serious, will be recorded on the AE Case Report Form, and if relevant, the Concomitant Medications Record in the eCRF will be updated. Severity and relationship to investigational product will be assessed by the PI as described in Table 4 and Table 5 respectively. Particular attention should be made to ensure no discrepancies between the AE and the SAE form (i.e., outcome, severity, relationship must be consistent).

Treatment-Emergent Adverse Events (TEAEs) are defined as AEs observed after first dose of investigational product.

Table 5: Relationship of AE to Treatment

Term	Definition	Clarification
No reasonable possibility (not related)	This category applies to AEs which, after careful consideration, are clearly due to extraneous causes (disease, environment, etc) or to AEs, which, after careful medical consideration at the time they are evaluated, are judged to be unrelated to the investigational product.	The relationship of an AE may be considered "no reasonable possibility" if it is clearly due to extraneous causes or if at least 2 of the following apply: • It does not follow a reasonable temporal sequence from the administration of the test drug. • It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. • It does not follow a known pattern of response to the test drug. • It does not reappear or worsen when the drug is re-administered.
Reasonable possibility (related)	This category applies to AEs for which, after careful medical consideration at the time they are evaluated, a connection with the test drug administration cannot be ruled out with certainty nor felt with a high degree of certainty to be related to the investigational product.	The relationship of an AE may be considered "reasonable possibility" if at least 2 of the following apply: • It follows a reasonable temporal sequence from administration of the drug. • It cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. • It disappears or decreases on cessation or reduction in dose. There are important exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists. • It follows a known pattern of response to the test drug.

7.2 Serious Adverse Events

An SAE is any AE occurring at any dose that suggests a significant hazard or side effect, regardless of the PI or Sponsor's opinion concerning the relationship to the investigational product and that result in, but may not be limited to, any of the following outcomes:

- Death (regardless of the cause)
- A life-threatening experience
- Inpatient hospitalisation or prolongation of existing hospitalisation (any inpatient hospital admission that includes a minimum of an overnight stay in a health care facility)
- A persistent or significant disability/incapacity
- A congenital anomaly or birth defect in an offspring of a study subject
- Significant medical events that may not result in death, be life-threatening, or require hospitalisation may be **serious** when, based upon appropriate medical judgement, they

may jeopardise the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Inpatient hospitalisation or prolongation of existing hospitalisation means that hospital inpatient admission and/or prolongation of hospital stay were required for treatment of AE, or that they occurred as a consequence of the event.

Hospitalisation for elective treatment of a pre-study condition (pre-treatment initiation) that did not worsen while on study and optional hospitalisations not associated with a clinical AE (e.g. elective cosmetic surgery) are not considered SAEs.

Significant medical events are those that may not be immediately life-threatening but may jeopardise the subject and may require intervention to prevent one of the other serious outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation. Such an AE will normally be considered serious by this criterion.

Progression of the cancer under study is not considered an AE unless it is considered to be drug-related by the PI.

In addition to the above criteria, AEs meeting any of the below criteria, although not serious per ICH definition, are reportable to the Sponsor for collection purposes as they are considered serious by Sponsor (in the same timeframe as SAEs, as 'significant medical events' to meet certain local requirements):

- A new cancer (that is not a condition of the study)
- Events associated with an excess dosing of investigational product above the recommended dose/per tumour size or dosing schedule.

Methods for follow-up for these criteria can be found in the Investigator Site File, or equivalent.

A **life-threatening** adverse drug experience is any AE that places the subject, in the view of the PI, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Any newly reported SAE, after Termination/Early Termination Visit, that is considered to be related to the investigational product or study participation, should be recorded and reported immediately to Sponsor or designee.

7.2.1 Definition of an Overdose for this Protocol and Reporting of Overdose to the Sponsor

An overdose in AGI-134 in humans cannot be defined as this is a first in human study.

In a GLP repeat-dose toxicology study in Cynomolgus monkeys, AGI-134 was well tolerated at doses exceeding the highest planned clinical dose (200 mg). The NOAEL was considered to be 50 mg/kg/dose (SC) (~5-fold higher than the highest expected clinical dose) and 10 mg/kg/dose (IV) (equivalent to the highest planned clinical dose that covers the highly vascularised nature of some solid tumour types and the risk encountered in the worst case of high systemic exposure).

No specific information is available on the treatment of an overdose of AGI-134. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

Refer to Safety Manual for Reporting of Overdose to Sponsor Instructions

7.2.2 Reporting Period of Serious Adverse Event

Once the subject has signed the Informed Consent Form (ICF), SAEs will be recorded up to 90 days following the last dose of study drug or until the subject initiates new anti-cancer therapy, whichever is earlier. All SAEs will be followed up for outcome.

Any SAEs, or follow-up to a SAE should be reported within 24 hours to the Sponsor. This includes death due to any cause other than progression of the cancer under study, whether or not related to the investigational product.

Additionally, any SAE, considered by a qualified physician to be related to the Sponsor's product that is brought to the attention of the PI at any time after the Termination/ET visit and up to 5 years, must also be reported immediately to the Sponsor.

7.2.3 Definition of an Unexpected Adverse Event

An unexpected adverse drug event is any AE, the specificity or severity of which is not consistent with information in the current IB for an unapproved investigational product.

Suspected Unexpected Serious Adverse Reaction (SUSAR) is a SAE assessed as unexpected by the Sponsor and that is judged by either the reporting PI or the Sponsor to have a reasonable causal relationship to the investigational product.

The sponsor will report all suspected unexpected serious adverse reactions, in compliance with regulatory guidelines and the Code of Federal Regulations, Title 21, Volume 5, revised as of April 1, 2018, 21 CFR 312.32 - IND safety reporting

7.2.4 Exceptions in the Reporting of SAE

There will be no exceptions in the reporting of SAEs in this study, as this is a first-in-man study.

7.2.5 Notification of SAE

7.2.5.1 Initial Notification of SAEs

An initial SAE Report Form must be completed and sent by fax/email to the Sponsor's MM within 24 hours of the PI's knowledge of the event. Any fatal or life-threatening event should be reported immediately, by phone, fax or email. Reporting SAEs to regulatory authorities and/or IRBs/IECs must comply with local regulations.

Medical Monitor

[redacted]

Pharmacovigilance Manager

[redacted]

Email: [redacted]

Fax: [redacted]

The initial SAE report will be followed within 24 hours by a completed SAE report including a sufficiently detailed narrative to allow for a medical assessment of the case, as well as copies of hospital case reports, results of applicable diagnostic tests, laboratory results, biopsy results, autopsy reports and other documents when requested and applicable.

Minimum Criteria for a Valid Initial SAE Case:

For regulatory purposes, initial SAE reports should be submitted to the Sponsor's MM or designee immediately and should include:

1. A suspected investigational product (whether or not it is suspected to be related investigational product)
2. An identifiable subject (i.e., study subject number)
3. An AE with the PI's assessment of seriousness
4. An identifiable reporting source (PI contact details)

Once sent, the SAE form and accompanying documentation should be placed in the SAE section of the Investigator's site file.

SAEs will be reported to the IRB/IEC and regulatory authorities as required by local regulations and International Conference on Harmonisation good clinical practice (ICH GCP) guidelines.

7.2.5.2 Follow-up of SAEs

Follow-up of all SAEs that occur during the study will continue until their satisfactory resolution or stabilisation. In outstanding cases, an SAE may be defined as "ongoing without further follow-up" if mutually agreed by the PI and Sponsor.

A Follow-up SAE Report Form must be completed by the site (marked as "Follow-up report") and sent to the Sponsor's MM within a reasonable timeframe (an SAE Follow-up report is required whether or not there is any additional information to the initial report).

The contact information for Follow-up SAE reporting is the same as for initial SAE reports (see above section).

As for the initial SAE report, once sent, the Follow-up SAE report and accompanying documentation should be placed in the SAE section of the Investigator's Site File.

7.2.6 Reporting of Pregnancy and Lactation to the Sponsor

Although pregnancy and lactation are not considered AEs, it is the responsibility of PIs or their designees to report any pregnancy or lactation in a subject or subjects' partner (spontaneously reported to them) that occurs during the study.

Pregnancies and lactations that occur after the ICF is signed but before Baseline must be reported by the PI and the subject should be excluded from the study.

Pregnancies and lactations that occur from the time of ICF signature through 120 days following Termination/ET visit, or 30 days following Termination/ET visit if the subject initiates new anti-cancer therapy, whichever is earlier, must be reported by the PI and captured in the eCRF. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion missed abortion, benign hydatidiform mole, blighted ovum, foetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (under Important Medical Event). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported to the Sponsor within 24 hours of the PI's knowledge of the event by fax or by email.

Email: [redacted]

Fax: [redacted]

8 STATISTICAL METHODOLOGY

8.1 Sample Size Consideration

As this is a Phase I/IIa study, the exact number of subjects enrolled will depend on the toxicity observed in each dose group and the number of dose groups required to reach MTD. If all four dose groups are required and if each dose group is expanded to 6 subjects (see Safety Assessment Section 8.3), then a total of 24 subjects will be enrolled into Part 1, the dose escalation, of the study. Part 2 is aiming to recruit approximately 35 subjects receiving at least one dose of AGI-134.

Analysis Sets

- Intention-To-Treat (ITT) analysis set: All enrolled subjects who received at least one dose of study drug.
- Per-Protocol (PP) analysis set: All enrolled subjects who completed the study according to the protocol without major protocol violations.
- Modified-Intention-To-Treat (mITT) analysis set: All enrolled subjects who receive at least one dose of investigational product and had post Baseline imaging assessment.
- Safety Population: All enrolled subjects who received at least one dose of study drug.

8.2 Statistical Methods

This section briefly describes the statistical methods to be used to analyse the data from the study. A detailed Statistical Analysis Plan (SAP) will present the methods in detail. If the methods of the SAP differ from the methods in this section, the methods of the SAP will prevail.

All measured variables and derived parameters will be listed individually and, if appropriate, summarised using descriptive statistics.

For categorical variables, summary tables will be provided giving sample size, absolute and relative frequency, and 95% confidence interval (CI) for proportions.

For continuous variables, summary tables will be provided giving sample size, arithmetic mean, standard deviation, median, minimum and maximum, appropriate percentiles, and 95% CIs for means.

Statistical test performed will be considered statistically significant if the P-value is less than [redacted].

The data will be analysed using the SAS ® version [redacted]).

The data will be analysed as described in the SAP.

Analysis will be performed for all subjects in the study, as well as for the pre-defined populations, as follows:

1. All patients in Part 1
2. All patients in Part 1 and 2

3. Part 2 patients from a specific sub-population with histologically confirmed advanced cutaneous metastatic melanoma who:
    1. Had only one previous treatment with CIP:
        a) Anti-PD-1 approved for melanoma, and/or
        b) Anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) therapy approved for melanoma, and
    2. Have remained on prior CPI treatment for at least 4 months or have stopped treatment earlier than that, due to toxicity rather than disease progression.

8.2.1 Efficacy Endpoints

8.2.1.1 Primary Endpoint

To assess the safety and tolerability of AGI-134 injected intratumourally, as well as determine the MTD and the recommended Part 2 dose (RP2D).

8.2.1.2 Secondary Endpoints

- To characterise the PK profile of AGI-134
- To assess the immune response to AGI-134 and to support the MoA
- To assess biomarkers that may serve as surrogates or predictors of clinical efficacy

8.2.1.3 Exploratory Endpoints

Exploratory endpoints will include the following efficacy endpoints:

- [redacted]

8.2.2 Analysis of Safety and Tolerability

Safety parameters (vital signs and laboratory results) will be summarised in listings and presented in tables as changes from Baseline.

ECG examinations results will be displayed in descriptive tables provide the frequency of subjects with abnormal findings for each schedule visit.

AEs will be coded using the latest version of MedDRA (currently version 24.0) and presented in tables by system organ class (SOC), HLT and preferred term. The worst severity grade of AEs, as determined by the latest version of the NCI-CTCAE (currently version 5.0), and their relationship to investigational product will be analysed by SOC, High Level Terms and Preferred Term.

The action taken, and outcome will also be analysed.

By-subject list of AEs, severity grade and relationship will be presented in time-sequence manner using the latest version of NCI-CTCAE (currently 5.0) with SOC, High Level terms and Preferred Term. The number and incidence of subjects experiencing toxicity will be tabulated by worst severity grade in each MedDRA term. The proportion of subjects experiencing toxicity $\geq$ Grade 3 for each type of toxicity will be calculated.

8.3 Ongoing Safety Assessment and Dose-Limiting Toxicity

AEs will be reported from the time of informed consent signature and up to 30 days after the last dose of study drug or until the initiation of new anti-cancer treatment, whichever is sooner. SAEs will be recorded up to 90 days following the last dose of study drug or until the subject initiates new anti-cancer therapy, whichever is earlier.

The period considered for dose-limiting toxicity (DLT) and safety assessment for the accelerated escalation is defined as the time from the first dose administration of AGI-134 and up to the end of the first cycle of therapy (i.e. Cycle 1 - Day 1 through Cycle 1 - Day 21). Data will be reviewed by the Safety Committee. The Safety Committee will be composed of the Study PI together with the Sponsor's MM and Pharmacovigilance Manager (PhV) or designee which will be responsible for the review of all the safety data at pre-defined time points during Part 1 and 2. Safety will be monitored throughout the study.

Beyond the DLT assessment period, data will be assessed continually on an ongoing basis. AEs reported beyond the DLT assessment period will be captured within the eCRF, however will not be considered for DLT assessment period. During Part 2 of the study, any specific AE that fits the DLT definition, occurring at a frequency of >30% will stop accrual. In this situation, the Safety Committee may recommend a lower dose level or dosing schedule change for the continuation of AGI-134 at the RP2D.

Sites will be required to contact the Sponsor within 24 hrs if subjects present with an AGI-134 related AE that is moderate or higher grade and clinically significant or experience DLTs during the DLT assessment period (see Section 3.1.1).

A subject that dropped out of the study will be included in all relevant data analyses and presentation as described in the statistical section.

8.3.1 Part 1 – Accelerated Dose Escalation of AGI-134

At any given dose, if one subject presents with a dose-limiting toxicity (DLT) or an event of moderate or higher severity assessed as related to AGI-134 and as clinically significant during the first cycle of treatment, the group of subjects will be expanded and the study will continue in a conventional "3+3" design. The expansion after an event of moderate severity will be to a total of 3 subjects and the expansion after a DLT will be to a total of 6 subjects, recruited in a stepwise manner of 3+3. Moderate injection site reactions are expected events with any injectable drug, as are moderate drug administration-related fever, nausea and vomiting. These will therefore will not be considered as a trigger for conversion to a "3+3" design. If the group of subjects was expanded due to a clinically significant related event that is not a DLT, and this expanded group, i.e. 3 subjects, showed no DLT events, then the study will continue to the next dose level enrolling 3 subjects.

If 1 out of 3 subjects presented with a DLT in the expanded cohort, a group of additional 3 subjects will be treated at the same dose level. If no more DLTs are reported at that dose, i.e. only 1 out of a total of 6 subjects has developed a DLT, the dose escalation will continue to the next level. At any given dose, if 2 or more out of 6 subjects experience a DLT, that dose level will be considered as the DLT dose. In such a case, 3 more subjects will be administered at the previous dose level, if there are less than 6 subjects already treated at that dose. MTD is defined as the highest dose level in which 6 subjects have been administered with and less than 2 instances of DLT.

When the first subject in each dose completes the first cycle of treatment, (each Cycle is 3 weeks long), the safety and tolerability will be assessed by a Safety Committee to decide whether to escalate the dose or to expand the group of subjects. When the MTD is reached or the 200 mg dose is found to be safe, whichever comes first, it will be defined as the RP2D and the study will continue to Part 2.

After completion of the first treatment cycle, subjects in Part 1 of the study will continue receiving intratumoural injection at the dose determined at Screening for 3 additional cycles. No intra-subject escalation will be allowed. The treatment regimen is one dose per cycle of AGI-134 every three weeks for up to 4 cycles.

8.3.2 Part 2 - AGI-134 at the RP2D

Safety interim analyses are planned after 3 subjects with deep injectable lesions have completed the first cycle with AGI-134 (i.e. Cycle 1 - Day 1 through to Cycle - Day 21). Sites will be required to contact the Sponsor within 24 hrs if a subject present with a DLT during the DLT assessment period (see Section 3.1.1).

During Part 2, 3 subjects will be evaluated after one cycle of injection into deep lesions. If one or more of the subjects experiences DLTs, a new assessment will be done after the additional 3 subjects with deep lesions injected are enrolled and treated (total 6 subjects). If no new DLTs are reported, the recruitment will continue as planned. If 2 or more subjects presented a DLT, the SC will assess the risk vs. benefit of the treatment and recommend whether or not the dose of AGI-134 should be reduced and/or the treatment schedule be changed (see Section 8.3).

Following the assessment of the first 3 patients with deep lesion injected or 6 patients if expansion is needed, the safety data will be evaluated by the Safety Committee. Evaluation by

the Safety Committee will be conducted every 6 months to review ongoing safety data, until the last patient last visit (LPLV).

8.3.3 Definition of Dose-Limiting Toxicity

A dose-limiting toxicity (DLT) is defined as a clinically significant AEs or abnormal laboratory value assessed as unrelated to disease progression, intercurrent illness or concomitant medications, occurring after at least one dose of investigational product during the DLT assessment period (see Section 3.1.1) and meeting any of the following criteria:

- CTCAE Grade 3 AST (SGOT) or ALT (SGPT) or bilirubin for ≥7 days
- CTCAE Grade 4 AST (SGOT) or ALT (SGPT) of any duration
- CTCAE Grade 3 or higher febrile neutropenia, neutropenia or thrombocytopenia
- CTCAE Grade 4 (life-threatening) vomiting or diarrhoea, regardless of response to management.
- CTCAE Grade 4 electrolyte abnormalities, regardless of response to management.
- All other, AEs CTCAE Grade 4.
- All other AEs Grade 3 with the following exceptions: Transient (less than 24 hours within injection time) nausea and vomiting, investigational product-related fever, electrolyte abnormalities (including K, Na, Cl, HCO3, Mg, Ca) anorexia and alopecia.

To be considered a DLT, the toxicity should be assessed as at least possibly related to the investigational product.

An AE must be clinically significant to be defined as a DLT (e.g. nausea and vomiting, investigational product-related fever, electrolyte abnormalities (including K, Na, Cl, HCO3, Mg, Ca) that are ≤ Grade 3 will not constitute a DLT).

9 ETHICS

9.1 Institutional Review Board or Independent Ethics Committee

Prior to initiation of the study, the PI will submit the study protocol and amendments, IB and amendments, ICF and any other documents that may be provided to the subject or any other documents requested by the IRB/IEC, for review and approval.

The names and affiliations of all members of the IRB/IEC must be provided to the PI and Agalimmune Ltd. In lieu of this, the IRB/IEC must certify that it has been officially authorised/recognised according to the national legislation.

The IRB/IEC must provide written approval of the study to keep in the Investigator Site File. Records of approval of all documents pertaining to this study, including the local Regulatory Authority, should be filed as such. The PI will not begin the study until the protocol, ICF and any other document provided to the subject have been approved by the IRB/IEC. The PI must agree to make any required progress reports to the IRB/IEC, as well as reports of SAEs, life-threatening conditions or death. The IRB/IEC will also be notified of Part 1 preliminary results.

9.2 Ethical Conduct of The Study

All clinical work conducted under this protocol is subject to ICH GCP (E6) guidelines. This includes an audit by Sponsor or its designee and inspection by health authority or IRB/IEC representatives at any time. The PI must agree to the inspection of study-related records by health authority representatives and/or audit by Sponsor or its designee.

The study will be conducted in accordance with Sponsor and/or designee's standards operating procedures (SOPs) and the following guidelines:

- GCP: Consolidated Guideline (International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use, May 1996).
- Declaration of Helsinki: Brazil, 2013.
- US Code of Federal Regulations (Title 21, CFR Part 11, 50, 54, 56 and 312) and/or EU Directives; and/or local country regulations and guidelines.

9.3 Subject Information and Consent

Prior to screening for the study, each subject will be informed in detail about the investigational products to be administered and the nature of the clinical investigation, including the risks and discomforts to be expected. The basic elements of informed consent, as specified by the US Government (21 CFR 50.25) and ICH GCP, will be followed. The subjects will also be instructed that they are free to withdraw their consent and discontinue their participation in the study at any time without prejudice. Written consent will be obtained from each subject to be involved in the clinical study by using the IRB/IEC approved ICF prior to the conduct of any study-related activity. A copy of the template ICF will be submitted together with this protocol and must be approved by the IRB/IEC prior to study commencement. Each subject will be given a copy of the written ICF, and each subject's chart will include the signed ICF for study participation. The

original subject signed and dated ICFs will be maintained per ICH record retention requirements in the Investigator Site File.

The initial ICF, any subsequent revised written ICF and any written information provided to the subject must receive the IRB/IEC's approval/favourable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the study. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

9.4 Subject Insurance

A product liability to cover against injury and damages arising from the use of investigational drug in this project is provided by the Sponsor for the total duration of the study covering the subjects and investigators in respect of the risks involved in conducting this study according to this protocol. The insurance policy will be filed in the Investigator Site File (ISF) or can be made available to the Investigator and to the IRB/IEC upon request.

Subjects will be insured through contract between an insurance company and the Sponsor.

9.5 Informing the General Practitioner

When required by local regulation, the PI will inform the subject's primary care physician of his/her participation in the study, by sending a letter to the physician.

9.6 Personal Data Protection

The Sponsor will comply with local regulations and with the principle of a subject's right to protection against invasion of privacy. Throughout this study, all subject data will be identified only by a subject identification number and subject initials and date of birth. The subject must be informed and consent to authorised personnel of the Sponsor, such as CRA, auditor, etc. and relevant health regulatory agencies having direct access to personal medical data to assure a high-quality standard of the study. At the subject's request, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare.

9.7 Safety Committee

A Safety Committee will be composed by the Study PI, the Sponsor Medical Monitor and pharmacovigilance associate. This committee will be responsible for the review of all the safety data at pre-defined time points in order to assess the safety of AGI-134. AEs will be monitored throughout the study.

During Part 1, every escalation dose will be assessed by this committee to decide whether to continue or to expand to a "3+3" design. If expanded, each dose will be assessed after recruitment of 3 subjects or more if >1 DLT is reported.

During Part 2, 3 subjects will be evaluated after one cycle of injection into deep lesions. If one or more of the subjects experiences DLTs, a new assessment will be done after the additional 3 subjects with deep lesions injected are enrolled and treated (total 6 subjects). If no new DLTs are reported, the recruitment will continue as planned. If 2 or more subjects presented a DLT, the SC will assess the risk vs. benefit of the treatment and recommend whether or not the dose of AGI-134 should be reduced and/or the treatment schedule be changed (see Section 8.3).

Following the assessment of the first 3 patient with deep lesion injected or 6 patients if expansion is needed, the safety data will be evaluated by the Safety Committee every 6 months to review ongoing safety data and until the last patient last visit (LPLV).

9.8 Protocol Exceptions and Deviations

Deviations from the protocol should be avoided, unless required for the safety of the subject. Protocol deviations, and, if possible, the reason for occurrence will be documented by the CRA in visit reports and will be included in the final clinical study report. The PI must report any protocol deviations to the Sponsor or the Sponsor's designee, should they occur. If required, the PI should also report deviations to the IRB/IEC in accordance with local regulations and within a reasonable time. No prospective waivers will be allowed for subjects who do not fulfil the inclusion/Exclusion Criteria.

9.9 Protocol Amendments

Changes to the protocol may be made only by the Sponsor (with or without consultation with the PI). All protocol amendments must be submitted to the IRB/IEC in accordance with local requirements and, if required, to the Regulatory Authority, either as an amendment or a notification. Approval for amendments must be received before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to study subjects, or when the changes involve only logistical or administrative aspects of the study. No approval is required for notifications.

9.10 Quality Control and Quality Assurance

The study will be conducted according to GCP as outlined by ICH Topic E6 step 5 guidelines. The Sponsor's SOPs will be followed to ensure that clinical studies are conducted and data are generated, documented and reported in compliance with the protocol, GCP and applicable regulatory requirements.

9.11 Audits and Inspections

The study may be audited according to the Sponsor's or its designee's Quality Assurance inspection programme. The purpose of the audit is to determine whether the study is being conducted and monitored in compliance with the study protocol and ICH GCP guidelines. Audit visit(s) will be arranged in advance with site personnel at a mutually acceptable time.

The PI should understand that source documents for this study should be made available to appropriately qualified personnel from the Sponsor or its designees or the Regulatory Authority inspectors after appropriate notification. The verification of the eCRF data must be made by direct inspection of source documents. The auditor may ask to visit the facilities where

laboratory samples are collected, where the study drug is stored and prepared and any other facility used during the study. These audits or inspections may take place at any time, during or after the study, and are based on the national regulations, as well as ICH guidelines.

9.12 Study Monitoring

Monitoring of the study, which is the responsibility of the Sponsor, may be delegated to a Contract Research Organisation (CRO) or an independent CRA. The CRA will advise the PI regarding the practical conduct of the study and maintaining compliance with the protocol, GCP and all applicable regulatory requirements.

Before study start, at the site initiation visit or at an Investigator's meeting, a Sponsor or CRO representative will review the protocol and eCRFs with the PI and site staff. The Sponsor/CRO will also be responsible for training study personnel in the study-specific procedures.

Throughout the course of the study, the CRA will oversee the conduct and the progress of the study by frequent contacts with the PI and site staff. Contact will include telephone calls and on-site visits. During the on-site visits, the eCRF will be reviewed for completeness and accuracy with corresponding source documents. As part of the visit, source documents will be made available for review by the CRA. The CRA will also perform drug accountability checks and will periodically review the Investigator Study File to ensure completeness of documentation in all respects of clinical study conduct.

Periodically, some or all of the facilities used in the study (e.g., local laboratory, pharmacy) may be visited. Monitoring visits will be arranged in advance with site personnel at a mutually acceptable time. Sufficient time must be allowed by the site personnel for the monitor to review eCRFs and relevant source documents. The PI should be available to answer questions or resolve data clarifications. The PI or delegated person will accompany the CRA during these on-site visits, cooperate in providing the documents for inspection and respond to enquiries.

The PI will ensure that the study subjects are aware of and consent to their personal information being scrutinised during the data verification process, as part of study-related monitoring, inspection and/or auditing, by properly authorised persons associated with Sponsor or by domestic and/or foreign regulatory authorities. However, the subject's participation and personal information will be treated as strictly confidential to the extent that the applicable law permits and will not be made publicly available.

Upon completion of the study, the study monitor will arrange for a final review of the study files after which the files should be secured for the appropriate time period according to Section 10.4.

9.13 Quality Laboratory Standards

Laboratory tests or evaluations described in this protocol will be conducted in accordance with quality laboratory standards as described in the SOPs of the central and local institution laboratories.

Before the study begins, where applicable, the laboratories to be used in the study will provide a list of the reference ranges for all laboratory tests to be undertaken and details of the method used for quality control. These will be held in the Investigator Site File and the Sponsor File. The methods employed for each assay should be available on request. Any change in the

laboratory, its procedures, references, values, etc. during the study must be notified promptly to the Sponsor.

Academic laboratories, if used in this study, may be exempt from some or all of the above requirements on a case by case basis.

9.14 Study Documentation

Study documents will include the following:

- Signed ICFs
- Source documents (e.g. subject files, medical notes)
- Investigator copies of the eCRFs and SAE reports
- Investigator Site File and its content
- Study manual (including imaging tip sheet)
- Laboratory Manual
- Study Pharmacy Manual (includes instructions for use)
- Training materials

Upon completion of the study, the CRA will perform a final review of the study files at the site after which the files should be secured for the appropriate time period according to the Sponsor's SOP and local regulations.

9.14.1 Source Documents

Source documents are original records in which raw data are first recorded. These may be office/clinic/hospital records, charts, diaries, x-rays, laboratory results, printouts, pharmacy records, care records or completed scales for each study subject. Source documents should be kept in a secure and limited-access area. All source documents must be accurate, clear, unambiguous, permanent and capable of being audited and inspected. They should be made available using a permanent form of recording (ink, typing, printing, optical disc etc). They should not be obscured by correcting fluid or have temporary attachments (such as removable self-stick notes). Source documents that are computer generated and stored electronically must be printed, signed and dated by the PI.

Source data for subjects registered to the study should indicate the date the ICF was signed, participation in a clinical study with the clinical protocol number and title, treatment number and evidence that inclusion/Exclusion Criteria have been met.

The PI will permit study-related monitoring, audits by or on behalf of the Sponsor, IRB/IEC review and regulatory inspections providing direct access to source data documents.

9.14.2 Recording of Data on the Electronic Case Report Form

The development of the electronic case report form (eCRF) will be the responsibility of the Sponsor or its designee.

All the pertinent data will be recorded on an eCRF. All eCRFs will be completed in English and will be reviewed by CRA for accuracy and completeness. All data pertaining to the visit should be recorded in the eCRF no later than five days after the completion of the visit. The PI is responsible for verifying that all data entries in the eCRFs are accurate and correct. The PI must sign the completed eCRF prior to its submission to the Sponsor.

A representative of the Sponsor or designee will instruct the PI and the site staff prior to the enrolment of the first subject and will train them on recording the findings into the eCRF system.

After the enrolment of the first subject, a CRA will periodically monitor the progress of the study. The study monitor will also have the ability to review query statuses remotely which may warrant more frequent contact with the PI and site staff. The PI will make available to the CRA the computer that accesses the eCRFs, source documents, signed consent forms and all other study-related documents. The PI is responsible for reviewing eCRFs, providing resolution to data queries generated by the CRA via the EDC system, providing missing or corrected data, and approving all changes performed on the data, and endorsing the subject data within the EDC system. This approval method will include applying an electronic signature, a uniquely assigned username and a password, that together would represent a traditional handwritten signature.

The PI will agree to the inspection of study-related records by the Sponsor, external auditor and/or health authority representatives.

9.14.3 Investigator Site File

All documents required for the conduct of the study as specified in the ICH GCP guidelines will be maintained by the PI in an orderly manner and made available for monitoring and/or auditing by the Sponsor/or designee and regulatory agencies.

9.15 Clinical Study Supplies

The Sponsor or designee will be responsible for supplying clinical study supplies to the sites. The PI will be responsible for the administration, inventory and accountability of all clinical study supplies provided to the site, exercising accepted medical and pharmaceutical practices. An accurate and timely record of the disposition of all clinical supplies must be maintained. The supplies and inventory record must be made available for inspection upon request. Upon completion or termination of the study, the PI will return the remaining clinical supplies along with a copy of the inventory record and a record of the clinical supplies returned to the Sponsor. A copy of these records should be maintained in the site study files. Under no circumstances will the PI allow the investigational products to be used other than as directed by this protocol.

Clinical study supplies include, but are not limited to: eCRFs, lab supplies, and investigational products.

9.16 Data Management

Data Management services will be provided by the Sponsor or designee. The Data Management system will be specified in the Data Management Plan.

After the data has been entered and verified, various edit checks will be performed to ensure the accuracy, integrity and validity of the database. These edit checks may include:

- Missing value checks
- Range checks
- Consistency checks
- Sequence checks
- Protocol adherence checks

Queries generated from these checks will be sent to the site for resolution, and the database will be updated to reflect query resolutions as appropriate.

AEs will be coded using the latest version of MedDRA (currently version 24.0). Prior and concomitant medications will be coded according to the World Health Organisation (WHO) Drug Dictionary.

10 STUDY ADMINISTRATION

10.1 Participating Centres

Approximately 15 sites will participate in the study. Additional sites may be added if needed.

10.2 Required Documents Prior to Study Initiation

Prior to the start of this study, all pre-investigational requirements must be met by the PI and site. These may include:

- Appropriate local health authority approval
- Documentation properly signed and dated by the required Investigator (i.e., documents required for submission to the local IRB/IECs or applicable regulatory authorities).
- Signed copy (original) of the approved protocol.
- Completed and signed statement of PI.
- A signed Clinical Study Agreement.
- Curriculum vitae for the PI and sub-Investigator (may be collected at site initiation visit).
- IRB/IEC name and address; and membership list (may be collected at site initiation visit).
- Letter of approval from the IRB/IEC for the protocol (identified by protocol title and number) and the ICF (identified by protocol title and number).
- Copy of the IRB/IEC-approved written ICF to be used in the study (that has also been approved by the Sponsor).

- Provisions for direct access to source/data documents if necessary for study-related monitoring, audits, IRB/IEC review and regulatory inspection.
- Name and location of the laboratory utilised for laboratory assays and other facilities conducting tests, as well as a copy of the laboratory certificate and list of normal laboratory ranges (may be collected at site initiation visit).

In case a laboratory certification is not available, a written statement as to how the laboratory complies with quality assurance should be provided.

Upon satisfactory receipt of all required regulatory documents, the Sponsor will arrange for investigational products to be delivered to the study site. Supply of all other study materials will be the responsibility of the Sponsor and/or designee. Subject entry should not begin until after the required regulatory documents are confirmed as received and the Investigator Meeting/Initiation Meeting has occurred. All personnel expected to be involved in the conduct of the study will undergo study initiation which will include review of study protocol, instructions for eCRF completion, AE reporting and overall responsibilities including those for drug accountability and study file maintenance.

The PI will be provided with an Investigator's file. This file should be used for all study-related documents. The PI will be responsible for keeping the Investigator Site File updated and ensuring that all required documents are filed. The file will be reviewed by the CRA during monitoring visits.

10.3 Study Completion

This study is expected to end when all required subjects have been enrolled and the last subject has completed the study and all query resolutions have been completed.

Data and materials that are required before the study can be considered complete and/or terminated include, but are not limited to:

- Laboratory findings, clinical data and all special test results from Screening through the end of the follow-up period
- eCRF properly completed by appropriate study personnel and signed by the PI
- Completed Drug Accountability Records
- Statement of outcome for each SAE reported
- Copies of protocol amendments and IRB/IEC as well as relevant health authority approval/notification (if applicable)
- Retention of Study Documents Statement

10.4 Retention of Study Records

The PI will retain copies of the approved protocol, completed eCRF, signed ICFs, relevant source documents and all other supporting documentation related to the project as defined in ICH-E6 Section 8 related to the project per ICH-E6 record retention requirements for at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product in a

secure and safe facility with limited-access. If the PI is unable to retain the study documents for the required amount of time, the Sponsor must be informed of the individual who will be assuming this responsibility.

The Sponsor will notify, in writing, the PI when the clinical study data may be discarded. The PI will take measures to prevent accidental or premature destruction of these documents.

These files must be made available for inspection upon reasonable request by authorised representatives of the Sponsor and/or the relevant regulatory agencies.

10.5 Confidentiality and Publication of Study Results

10.5.1 General

All data and information supplied by or on behalf of the Sponsor or otherwise acquired or obtained by any Research Institution, the PI and other investigators ("**Recipients**") in any manner, in connection with or in performance of this study, is considered "**Confidential Information**." This Confidential Information includes, but is not limited to, the IB, this protocol and any information relating thereto, eCRFs and other scientific data, information relating to Sponsor's Investigational Product and treatment methodology and information relating to Sponsor's (or its affiliates') commercial, technical and financial information, research technology, products, inventions, trade secrets and research and development. The results produced in performance of the study and any data, information or other material collected or generated in the course of performing the study shall be promptly disclosed to Sponsor in full in writing and are also considered Confidential Information. This Confidential Information shall be the sole property of the Sponsor.

Except for Publishable Results (defined below) to the extent it may be published under Section 10.5.2, throughout the duration of the study and after its completion, Recipients shall

(i) not disclose Confidential Information to others without the written consent of the Sponsor, except to those of its employees who have a need to know the Confidential Information in order to enable Recipients' to fulfil their obligations hereunder, and where such employees are bound by written contractual obligations covering Confidential Information that are no less restrictive or protective than those contained herein, *provided that* Recipients shall remain liable for any disclosure or use of Confidential Information by such employees;

(ii) use the same degree of care to preserve confidentiality of Confidential Information as they use for their own information of like nature, which shall not be less than reasonable degree of care; and

(iii) not use Confidential Information for any purpose except in the performance of this study. Promptly at Sponsor's request, or upon completion of the study, Recipients will discontinue use and return to Sponsor or destroy, in accordance with Sponsor's instructions, all copies or other manifestations of Confidential Information that may be in their possession or control, except to the extent expressly required hereunder and to comply with Applicable Laws (defined below).

Should a Recipient be required to disclose Confidential Information pursuant to law, regulation, judicial or administrative order or request by a governmental or other entity authorised by law to make such request, Recipient shall (i) promptly notify Sponsor prior to such disclosure,

(ii) cooperate with Sponsor and provide assistance in seeking a protective order or other suitable protection with respect to the Confidential Information, and (iii) only disclose such Confidential Information to the extent pursuant to said law, regulation, judicial or administrative order, or request by a governmental or other authorised entity.

At the subject's request, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare. The personal physician will be notified by site personnel of subject participation in the study.

10.5.2 Published Data

The original eCRFs and the data generated from the eCRFs or otherwise obtained during the study under this study protocol will become the property of the Sponsor. Publication of the results of this study ("**Publishable Results**") in an appropriate peer-reviewed journal after the conclusion of the study ("**Publication**") made be made as provided herein. Publication must be undertaken in a responsible and ethical manner, taking into account relevant external standards regarding the manner and content of scientific, technical and medical publications and in subject to applicable laws, rules, regulations, policies and guidelines ("**Applicable Laws**"). Authorship will be determined by mutual agreement between Sponsor and PI. Sponsor shall be mentioned in all Publications unless contrary instruction is given by Sponsor. Review and comment by Sponsor authorised personnel on draft abstracts and manuscripts for Publication or presentation is required prior to publication or presentation. Authors shall submit a copy of any abstracts, manuscripts or other material proposed for publication or presentation ("**Draft Publications**") to the Sponsor for its approval no fewer than sixty (60) days prior to the intended date of submission of such Draft Publications to any journal, publisher, and/or third party. The Sponsor has the right, at its discretion (a) to evaluate Draft Publications for accuracy and concurrence regarding data, evaluations, and conclusions, (b) to provide an opportunity for Sponsor to share with the Investigator(s) any new or unpublished information of which he or she may be unaware, (c) to ensure that no Confidential Information or other Sponsor proprietary information is being utilised and has been included, and (d) evaluate Draft Publications to determine if patent applications need to be filed on any information disclosed therein.

If the Sponsor determines that such Draft Publication contains Confidential Information or could otherwise be detrimental to Sponsor's intellectual property interest or have other adverse effects on its business, and notifies PI of its determination, the PI, Research Institutions and other Investigators/authors shall remove such Confidential Information from the Draft Publication or at Sponsor's election, modify it to remove language that is detrimental to Sponsor's intellectual property or other interests, and refrain from submitting such Draft Publication to a journal, publisher and/or other third party for additional ninety (90) days from Sponsor's notification to allow for filing of patent applications or the taking of such other measures as Sponsor deems appropriate to establish, preserve and protect its intellectual property or other interests. Principal Investigator, other Investigators and Research Institutions further agree to redact or modify those sections of the draft Publication which the Sponsor in good faith determines falls within (a) to (d) above.

11 REFERENCES

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12 APPENDICES

APPENDIX A: Schedule of Assessments

Table 6: Schedule of Assessment - Part 1: Accelerated Dose Escalation of AGI-134

Study Period:	**Screening Phase**	**Baseline Assessments**	**3-Week Cycles**												**9-Week F.U Cycles**	**Termination Visit**	**Long-Term Follow-up**
Treatment Cycle:	**Screening (Visit 1)**	**Pre-Dose**	**Cycle 1**			**Cycle 2**			**Cycle 3**			**Cycle 4**			**Weeks 18, 27, 36 & 45**	**Week 54 or early Termination**	**Survival Follow-up call**
Days from injection (Safety assessment follow-up)	**-28**	**0**	**1**	**8**	**15**	**1**	**8**	**15**	**1**	**8**	**15**	**1**	**8**	**15**			
Scheduling Window (Days) unless specified otherwise	**-28 to 0**	**-3 to 1 Pre-dose**	**+3**			**±3**			**±3**			**±3**			**±3**	**±3**	**Every 9 weeks (±7 days)**
Informed Consent	X																
Inclusion/Exclusion Criteria	X	X[1]															
Demographics and Medical History	X																
Disease history (including genetic information, dMMR/MSI and Tumour Mutational status)	X																
Disease treatment history	X																
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Review Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
12-Lead Electrocardiogram (ECG) local reading	X		X[2]			X[2]			X[2]			X[2]				X	
12-Lead Electrocardiogram (ECG) central reading			X[3]									X[3]					
Full Physical Examination	X															X	

Study Period:	**Screening Phase**	**Baseline Assessments**	**3-Week Cycles**												**9-Week F.U Cycles**	**Termination Visit**	**Long-Term Follow-up**
Treatment Cycle:	**Screening (Visit 1)**	**Pre-Dose**	**Cycle 1**			**Cycle 2**			**Cycle 3**			**Cycle 4**			**Weeks 18, 27, 36 & 45**	**Week 54 or early Termination**	**Survival Follow-up call**
Days from injection (Safety assessment follow-up)	**-28**	**0**	**1**	**8**	**15**	**1**	**8**	**15**	**1**	**8**	**15**	**1**	**8**	**15**			
Scheduling Window (Days) unless specified otherwise	**-28 to 0**	**-3 to 1 Pre-dose**	**+3**			**±3**			**±3**			**±3**			**±3**	**±3**	**Every 9 weeks (±7 days)**
Symptoms Directed Physical Examination		X	X[4]	X	X	X[4]	X	X	X[4]	X	X	X[4]	X	X			
Examination of injection sites			X[5]	X	X	X[5]	X	X	X[5]	X	X	X[5]	X	X	X	X	
Vital Signs (blood pressures, pulse rate, O_2 sat., oral temperature (°C), respiratory rate)	X		X[7]	X	X	X[8]	X	X	X[8]	X	X	X[7]	X	X		X	
Weight	X															X	
Height	X																
ECOG performance status	X		X			X			X			X			X	X	
Photography pictures of lesions that are Superficial			X[5]	X	X	X[5]	X	X	X[5]	X	X	X[5]	X	X	X	X	
Review general instructions before study drug administration			X			X			X			X					
AGI-134 administration[9]			X			X			X			X					

Study Period:	**Screening Phase**	**Baseline Assessments**	**3-Week Cycles**												**9-Week F.U Cycles**	**Termination Visit**	**Long-Term Follow-up**
Treatment Cycle:	**Screening (Visit 1)**	**Pre-Dose**	**Cycle 1**			**Cycle 2**			**Cycle 3**			**Cycle 4**			**Weeks 18, 27, 36 & 45**	**Week 54 or early Termination**	**Survival Follow-up call**
Days from injection (Safety assessment follow-up)	**-28**	**0**	**1**	**8**	**15**	**1**	**8**	**15**	**1**	**8**	**15**	**1**	**8**	**15**			
Scheduling Window (Days) unless specified otherwise	**-28 to 0**	**-3 to 1 Pre-dose**	**+3**			**±3**			**±3**			**±3**			**±3**	**±3**	**Every 9 weeks (±7 days)**
Local Laboratory[10]																	
Pregnancy Test – Serum or Urine[11]	X	X				X						X				X	
FSH screening for women who reported menopause less than two years.	X																
Disease markers according to subject's disease	X					X						X					
HPV status for HNSCC[12]	X																
PT/INR and aPTT	X	X				X			X			X					
CBC with Differential	X	X	X[13]	X	X	X			X			X				X	
Biochemistry Panel	X	X	X[13]	X	X	X			X			X				X	
T3, free T4, and TSH	X					X						X				X	
HIV, HBV and HCV serology	X																
Urinalysis	X	X				X						X					

Study Period:	**Screening Phase**	**Baseline Assessments**	**3-Week Cycles**												**9-Week F.U Cycles**	**Termination Visit**	**Long-Term Follow-up**
Treatment Cycle:	**Screening (Visit 1)**	**Pre-Dose**	**Cycle 1**			**Cycle 2**			**Cycle 3**			**Cycle 4**			**Weeks 18, 27, 36 & 45**	**Week 54 or early Termination**	**Survival Follow-up call**
Days from injection (Safety assessment follow-up)	**-28**	**0**	**1**	**8**	**15**	**1**	**8**	**15**	**1**	**8**	**15**	**1**	**8**	**15**			
Scheduling Window (Days) unless specified otherwise	**-28 to 0**	**-3 to 1 Pre-dose**	**+3**			**±3**			**±3**			**±3**			**±3**	**±3**	**Every 9 weeks (±7 days)**
Central Laboratory																	
Blood for Anti-Gal Antibodies complement activation and cytokines		X								X[14]						X	
Blood for PBMC Isolation		X								X[14]					X[19]	X	
Blood for Anti-Gal IgE	X								X[4]								
HLA haplotyping		X															
Blood for immunophenotyping by FACS		X								X[14]					X[19]	X	
Blood for RNA/ DNA		X								X[14]					X[19]		
Blood for PK			X[15]			X[16]			X[16]			X[14,15]					
Biopsies																	
Biopsy of injected lesion[9]		X[17]								X[18]					X[19]		
Biopsy for un-injected lesion[9]		X[17]								X[18]					X[19]		
Disease Assessment																	
Tumour Imaging[20]	X										X				X	X	
RECIST 1.1 assessment	X										X[4]				X	X	

Study Period:	**Screening Phase**	**Baseline Assessments**	**3-Week Cycles**												**9-Week F.U Cycles**	**Termination Visit**	**Long-Term Follow-up**
Treatment Cycle:	**Screening (Visit 1)**	**Pre-Dose**	**Cycle 1**			**Cycle 2**			**Cycle 3**			**Cycle 4**			**Weeks 18, 27, 36 & 45**	**Week 54 or early Termination**	**Survival Follow-up call**
Days from injection (Safety assessment follow-up)	**-28**	**0**	**1**	**8**	**15**	**1**	**8**	**15**	**1**	**8**	**15**	**1**	**8**	**15**			
Scheduling Window (Days) unless specified otherwise	**-28 to 0**	**-3 to 1 Pre-dose**	**+3**			**±3**			**±3**			**±3**			**±3**	**±3**	**Every 9 weeks (±7 days)**
Post-study Anti-Cancer Therapy Status																X[21]	X
Survival Status																X[21]	X

1. Inclusion/Exclusion Criteria should be re-assessed at Baseline prior to final eligibility confirmation
2. A 12-lead ECG will be recorded at each cycle (1-4) prior to AGI-134 administration (up to 1 hour) and 2 hours after dose (±30 minutes). In cycle 1 and 4 when a Holter device is connected a printout should be extracted from the device at the appropriate timepoints
3. In Cycle 1 and 4 a 12-lead ECG holter recording device will be connected to the subject 1-2 hours prior to AGI-134 administration and record ECG consecutively for at least 24 hours after AGI-134 administration
4. Pre-dose only
5. To be performed up to 1 hour prior to AGI-134 administrations and 1 hour post AGI-134 administration.
6. On Cycle 1 and Cycle 4, to be performed also 72 hours post AGI-134 administration
7. Vital signs will be collected up to 1 hour pre-dose and 0.5, 1, 24, 36, 48 and 72 hrs post AGI-134 administration
8. Vital signs will be collected prior to AGI-134 administration and 2 hours post dose
9. Refer to "Administration AGI-134 and Tumour Biopsy Procedures in Study AGI-134.FIM.101" for detailed information
10. All laboratory assessment should be performed before investigation product administration unless otherwise specified. Coagulation test results should be available before AGI-134 injections and biopsies
11. Pregnancy test in Screening and Termination must be serum. At other visits should be a urine test
12. HPV status assessment should be performed at local lab in case no available status from the time of HNSCC diagnosis
13. To be drawn 72 hours post AGI-134 administration
14. To be drawn on Cycle 3, during week 8 (concomitant with biopsies)
15. To be drawn up to 1 hour prior to AGI-134 administration and 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48 and 72 hrs post AGI-134 administration
16. To be drawn up to 1 hour prior to AGI-134 administration and 2 hours post dose
17. The biopsies can be performed on the day of the first dose and up to 3 days prior
18. The biopsies should be performed on Cycle 3, during week 8

19. Optional, on week 27
20. CT or MRI (according to Imaging Manual)
21. Only for Early Termination visit

Table 7: Schedule of Assessment - Part 2: AGI-134 at the RP2D

	Screening Visit	Baseline Visit	Core Study									Long-Term Follow- up
			Cycle 1				Cycle 2	Cycle 3	Cycle 4	Visits	Termination Visit/Early Termination Visit	
Days	**0**	**0**	**Day 1**	**Day 3**	**Day 8**	**Day 15**						
Weeks	**0**	**0**	**1**	**1**	**2**	**3**	**4-6**	**7-9**	**10-12**	**18, 27, 36 & 45**	**54**	**Every 9 weeks**
Scheduling Window (Days) ***unless specified otherwise***	**-28 to 0**	**-3 to Day 1 (Pre-dose)**	**0**	**±1**	**+3**	**+3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±7**
Informed Consent	X											
Inclusion/Exclusion Criteria	X	X[1]										
Demographics and Medical History	X											
Disease history (including genetic information, dMMR/MSI/TMB)	X											
Disease treatment history	X											
Prior and Concomitant Medication Review	X	X	X		X	X	X	X	X	X	X	
Review Adverse Events	X	X	X		X	X	X	X	X	X[24]	X[24]	X[24]
12-Lead Electrocardiogram (ECG) local reading	X		X[2]				X[2]	X[2]	X[2]		X	
12-Lead Electrocardiogram (ECG) central reading			X[3]					X[3]				

	Screening Visit	**Baseline Visit**	**Core Study**									**Long-Term Follow- up**
			Cycle 1				**Cycle 2**	**Cycle 3**	**Cycle 4**	**Visits**	**Termination Visit/Early Termination Visit**	
Days	**0**	**0**	**Day 1**	**Day 3**	**Day 8**	**Day 15**						
Weeks	**0**	**0**	**1**	**1**	**2**	**3**	**4-6**	**7-9**	**10-12**	**18, 27, 36 & 45**	**54**	**Every 9 weeks**
Scheduling Window (Days) ***unless specified otherwise***	**-28 to 0**	**-3 to Day 1 (Pre-dose)**	**0**	**±1**	**+3**	**+3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±7**
Full Physical Examination	X										X	
Symptoms Directed Physical Examination		X	X[4]		X	X	X[4]	X[4]	X[4]			
Examination of injection sites			X[5]	X[6]	X	X	X[5]	X[6]	X[5]	X	X	
Vital Signs (blood pressures, pulse rate, O_2 sat., oral temperature (°C), respiratory rate)	X		X[7]		X	X	X[8]	X[7]	X[8]		X	
Weight	X										X	
Height	X											
ECOG performance status	X		X				X	X	X	X	X	
Photography of superficial injected and non-injected lesions			X[5]	X[6]	X	X	X[5]	X[6]	X[5]	X	X	
Review general instructions before study drug administration			X				X	X	X			
AGI-134 administration[9]			X				X	X	X			

	Screening Visit	**Baseline Visit**	**Core Study**									**Long-Term Follow- up**
			Cycle 1				**Cycle 2**	**Cycle 3**	**Cycle 4**	**Visits**	**Termination Visit/Early Termination Visit**	
Days	**0**	**0**	**Day 1**	**Day 3**	**Day 8**	**Day 15**						
Weeks	**0**	**0**	**1**	**1**	**2**	**3**	**4-6**	**7-9**	**10-12**	**18, 27, 36 & 45**	**54**	**Every 9 weeks**
Scheduling Window (Days) ***unless specified otherwise***	**-28 to 0**	**-3 to Day 1 (Pre-dose)**	**0**	**±1**	**+3**	**+3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±7**
Local Laboratory[10]												
Pregnancy Test – Serum or Urine[11]	X	X					X		X		X	
FSH screening for women who reported menopause less than two years	X											
Disease markers according to subject's disease	X						X		X			
HPV status for relevant indications[12]	X											
PT/INR and aPTT	X	X					X[4]	X[4]	X[4]			
CBC with Differential	X	X		X	X	X	X[4]	X[4]	X[4]		X	
Biochemistry Panel	X	X		X	X	X	X[4]	X[4]	X[4]		X	
T3, free T4, and TSH	X						X		X		X	
HIV, HBV and HCV serology	X											

	Screening Visit	**Baseline Visit**	**Core Study**									**Long-Term Follow- up**
			Cycle 1				**Cycle 2**	**Cycle 3**	**Cycle 4**	**Visits**	**Termination Visit/Early Termination Visit**	
Days	**0**	**0**	**Day 1**	**Day 3**	**Day 8**	**Day 15**						
Weeks	**0**	**0**	**1**	**1**	**2**	**3**	**4-6**	**7-9**	**10-12**	**18, 27, 36 & 45**	**54**	**Every 9 weeks**
Scheduling Window (Days) ***unless specified otherwise***	**-28 to 0**	**-3 to Day 1 (Pre-dose)**	**0**	**±1**	**+3**	**+3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±7**
Urinalysis	X	X					X		X			
Central Laboratory[25]												
Blood for Anti-Gal IgE	X							X[4]				
CH50			X[4]									
C5a			X[15]				X[17]	X[15]	X[17]			
Flow cytometry (FACS)			X[13]				X[4]	X[13]			X	
Cytokines			X[14]	X				X[14]				
Anti-Gal antibodies IgG/IgM			X		X		X[4]	X[4]	X[4]		X	
Anti-Gal sub classing			X[4]				X[4]	X[4]	X[4]		X	
PBMCs isolation + cryopreservation			X[4]	X			X[4]	X[4]			X	
HLA and MHCII haplotyping			X[4]									
DNA isolation (MSI/dMMR/TMB,WES) blood			X[4]									

	Screening Visit	**Baseline Visit**	**Core Study**									**Long-Term Follow- up**
			Cycle 1				**Cycle 2**	**Cycle 3**	**Cycle 4**	**Visits**	**Termination Visit/Early Termination Visit**	
Days	**0**	**0**	**Day 1**	**Day 3**	**Day 8**	**Day 15**						
Weeks	**0**	**0**	**1**	**1**	**2**	**3**	**4-6**	**7-9**	**10-12**	**18, 27, 36 & 45**	**54**	**Every 9 weeks**
Scheduling Window (Days) ***unless specified otherwise***	**-28 to 0**	**-3 to Day 1 (Pre-dose)**	**0**	**±1**	**+3**	**+3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±7**
TCR Sequencing blood			X[4]					X[4]			X	
RNA isolation (Gene expression/sequencing) blood			X[4]	X				X[18]			X	
Blood for PK (AGI-134 and metabolites)			X[16]				X[17]	X[16]	X[17]			
Biopsies[9]												
Biopsy of injected lesion		X[19]		X[20]				X[21]			X[22]	
Biopsy for un-injected lesion		X[19]						X[21]			X[22]	
Disease assessment												
Tumour Imaging[23]	X							X		X	X	
RECIST 1.1 assessment	X							X		X	X	
Post-study Anti-Cancer Therapy Status											X	X
Disease and Survival Status											X	X

1. Inclusion/Exclusion Criteria should be re-assessed at Baseline prior to final eligibility confirmation.
2. A 12-lead ECG will be recorded at each cycle (1-4) prior to AGI-134 administration (up to 1 hour) and 2 hours after dose (±30 minutes). In Cycle 1 and 3, when a Holter device is connected, a printout should be extracted from the device at the appropriate timepoints.
3. In Cycle 1 and 3, a 12-lead ECG Holter recording device will be connected to the subject 1-2 hours prior to AGI-134 administration and record ECG consecutively for at least 24 hours after AGI-134 administration.
4. Pre-dose
5. To be performed up to 1 hour prior and 1 hour post AGI-134 administration.
6. To be performed 48-72 hours post AGI-134 administration (prior to tumour biopsy if applicable)
7. Vital signs will be collected up to 1 hour pre-dose and 0.5, 1, 24, 36, 48 and 72 hrs post AGI-134 administration
8. Vital signs will be collected prior to AGI-134 administration and 2 hours post dose
9. AGI-134 should be administered on Day 1 of each Cycle. Refer to "Administration AGI-134 and Tumour Biopsy Procedures in Study AGI-134.FIM.101" for detailed information
10. All laboratory assessment should be performed before investigational product administration, unless otherwise specified.
11. Pregnancy test in Screening and Termination Visit must be serum. At other visits a urine test.
12. HPV status assessment should be performed at local lab in case no available status from the time of diagnosis.
13. To be drawn pre-dose and 24 hours post AGI-134 administration
14. To be drawn pre-dose and at 2,6,12, and 24 hours post AGI-134 administration
15. To be drawn pre-dose and at 2 and 6 hours post AGI-134 administrations
16. To be drawn up to 1 hour prior to AGI-134 administration and 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48 and 72 hrs post AGI-134 administration
17. To be drawn up to 1 hour prior to AGI-134 administration and 2 hours post dose
18. To be drawn 24 hours post AGI-134 administration
19. The biopsies can be performed on the day of the first dose and up to 7 days prior AGI-134 Injection
20. Superficial only between 48h±24 hours post AGI-134 administration
21. If done on the same day of AGI-134 administration, biopsy should be done prior to injection. If not on the same day, a window of -7 days prior AGI-134 administration is allowed
22. In case of Early Termination occurring prior to Cycle 3, every effort must be made to perform biopsies on the ET visit Two biopsies should be taken.
23. CT or MRI (according to Imaging Manual) or superficial lesion measurement (depending on to the tumour type). In Cycle 3, to be done between Week 9 and Cycle 4 – Day 1 (before injection of AGI-134).
24. AEs will be recorded up to 30 days after the last dose of study drug, and SAEs up to 90 or until the initiation of new anti-cancer therapy, whichever is sooner.
25. For detailed time window for sample collection refer to the Laboratory Manual

APPENDIX B: RECIST v1.1

RESPONSE EVALUATION CRITERIA IN SOLID TUMOURS, VERSION 1.1 (RECIST V1.1)

Selected sections from the Response Evaluation Criteria in Solid Tumours, Version 1.1 (RECIST v1.1), (Eisenhauer et al. 2009) are presented below, with slight modifications from the original publication and the addition of explanatory text as needed for clarity.

1 TUMOUR MEASURABILITY

At screening, tumour lesions/lymph nodes will be categorised as measurable or non-measurable as described below. All measurable and non-measurable lesions should be assessed at Screening and at subsequent protocol-specified tumour assessment timepoints. Additional assessments may be performed as clinically indicated for suspicion of progression.

1.1 Measurable Lesions

1.1.1 Tumour Lesions

Tumour lesions must be accurately measured in at least one dimension (longest dimension in the plane of measurement is to be recorded) with a minimum size as follows:

- 10 mm by computed tomography (CT) or magnetic resonance imaging (MRI) scan (CT/MRI scan slice thickness/interval ≤ 5 mm)
- 10-mm calliper measurement by clinical examination (lesions that cannot be accurately measured with callipers should be recorded as non-measurable)
- 20 mm by chest X-ray

1.1.2 Malignant Lymph Nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in the short axis when assessed by CT scan (CT scan slice thickness recommended to be ≤ 5 mm). At Baseline and follow-up, only the short axis will be measured and followed. Additional information on lymph node measurement is provided below (see "Identification of Target and Non-Target Lesions" and "Calculation of Sum of Dimensions").

1.2 Definition of Non-Measurable Lesions

Non-measurable tumour lesions encompass small lesions (longest dimension < 10 mm or pathological lymph nodes with short axis ≥ 10 mm but < 15 mm) as well as truly non-measurable lesions. Lesions considered truly non-measurable include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, peritoneal spread, and abdominal mass/abdominal organomegaly identified by PE that is not measurable by reproducible imaging techniques.

1.3 Special Considerations Regarding Lesion Measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment, as outlined below.

1.3.1 Bone Lesions

- Bone scans, PET tomography scans, and plain films are not considered adequate imaging techniques for measuring bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions with identifiable soft tissue components that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered measurable lesions if the soft tissue component meets the definition of measurability described above.
- Blastic bone lesions are non-measurable.

1.3.2 Cystic Lesions

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered malignant lesions (neither measurable nor non-measurable) because they are, by definition, simple cysts.
- Cystic lesions thought to represent cystic metastases can be considered measurable lesions if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same subject, these are preferred for selection as target lesions.

1.3.3 Lesions with Prior Local Treatment

- Tumour lesions situated in a previously irradiated area or in an area subjected to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression in the lesion.

2 METHODS FOR ASSESSING LESIONS

All measurements should be recorded in metric notation, using calipers if clinically assessed. All Baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterise each identified and reported lesion at Baseline and during the study. Imaging-based evaluation should always be the preferred option.

2.1 Clinical Lesions

Clinical lesions will only be considered measurable when they are superficial and ≥ 10 mm in dimension as assessed using calipers (e.g., skin nodules). For the case of skin lesions, documentation by colour photography, including a ruler to estimate the size of the lesion, is suggested.

2.2 Chest X-Ray

Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, because CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

2.3 CT and MRI Scans

CT is the best currently available and reproducible method to measure lesions selected for response assessment. In this guideline, the definition of measurability of lesions on CT scan is based on the assumption that CT slice thickness is ≤ 5 mm. When CT scans have slice thickness of > 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable.

If prior to enrolment, it is known that a patient is unable to undergo CT scans with intravenous (IV) contrast because of allergy or renal insufficiency, the decision as to whether a non-contrast CT or MRI (without IV contrast) will be used to evaluate the patient at Baseline and during the study should be guided by the tumour type under investigation and the anatomic location of the disease. For patients who develop contraindications to contrast after baseline contrast CT is done, the decision as to whether non-contrast CT or MRI (enhanced or non-enhanced) will be performed should also be based on the tumour type and the anatomic location of the disease and should be optimised to allow for comparison with prior studies, if possible. Each case should be discussed with the radiologist to determine if substitution of these other approaches is possible and, if not, the patient should be considered not-evaluable from that point forward. Care must be taken in measurement of target lesions and interpretation of non-target disease or new lesions on a different modality, because the same lesion may appear to have a different size using a new modality.

2.4 Endoscopy, Laparoscopy, Ultrasound, Tumour Markers, Cytology, Histology

Endoscopy, laparoscopy, ultrasound, tumour markers, cytology, and histology cannot be utilised for objective tumour evaluation.

3 ASSESSMENT OF TUMOUR BURDEN

To assess objective response or future progression, it is necessary to estimate the overall tumour burden at Baseline and use this as a comparator for subsequent measurements.

3.1 Identification of Target and Non-Target Lesions

When more than one measurable lesion is present at Baseline, all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at Baseline. This means that, for instances in which patients have only one or two organ sites involved, a maximum of two lesions (one site) and four lesions (two sites), respectively, will be recorded. Other lesions (albeit measurable) in those organs will be considered non-target lesions.

Target lesions should be selected on the basis of their size (lesions with the longest dimension) and be representative of all involved organs, but in addition should lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement, in which circumstance the next largest lesion that can be measured reproducibly should be selected.

Lymph nodes merit special mention because they are normal anatomical structures that may be visible by imaging even if not involved by tumour. As noted above, pathological nodes that are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥ 15 mm by CT scan. Only the short axis of these nodes will contribute to the Baseline sum. The short axis of the node is the dimension normally used by radiologists to judge if a node is involved by solid tumour. Lymph node size is normally reported as two dimensions in the plane in which the image is obtained (for CT, this is almost always the axial plane; for MRI, the plane of acquisition may be axial, sagittal, or coronal). The smaller of these measures is the short axis. For example, an abdominal node that is reported as being 20 mm × 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement. All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be considered non-target lesions. Nodes that have a short axis of < 10 mm are considered non-pathological and should not be recorded or followed.

All lesions (or sites of disease) not selected as target lesions (measurable or non-measurable), including pathological lymph nodes, should be identified as non-target lesions and should also be recorded at Baseline. Measurements are not required. It is possible to record multiple non-target lesions involving the same organ as a single item on the Case Report Form (CRF) (e.g., "multiple enlarged pelvic lymph nodes" or "multiple liver metastases").

3.2 Calculation of Sum of Dimensions

A sum of the dimensions (longest dimension for non-lymph node lesions, short axis for lymph node lesions) will be calculated for all target lesions at baseline and at each subsequent tumour assessment as a measure of tumour burden.

3.2.1 Measuring Lymph Nodes

Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the node regresses to < 10 mm during the study. Thus, when lymph nodes are included as target lesions, the sum of dimensions may not be zero even if CR criteria are met, because a normal lymph node is defined as having a short axis of < 10 mm.

3.2.2 Measuring Lesions That Become Too Small to Measure

During the study, all target lesions (lymph node and non-lymph node) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g., 2 mm). However, sometimes lesions or lymph nodes that are recorded as target lesions at Baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measurement and may report them as being too small to measure. When this occurs, it is important that a value be recorded on the CRF, as follows:

- If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm.
- If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned and "too small to measure" should be ticked. (Note: It is less likely that this rule will be used for lymph nodes because they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well, and "too small to measure" should also be ticked).

To reiterate, however, if the radiologist is able to provide an actual measurement, that should be recorded, even if it is < 5 mm, and in that case "too small to measure" should not be ticked.

3.2.3 Measuring Lesions That Split or Coalesce on Treatment

When non–lymph node lesions fragment, the longest dimensions of the fragmented portions should be added together to calculate the sum of dimensions. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal dimension measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest dimension in this instance should be the maximum longest dimension for the coalesced lesion.

3.2.4 Evaluation of Non-Target Lesions

Measurements are not required for non-target lesions, except that malignant lymph node non-target lesions should be monitored for reduction to < 10 mm in short axis. Non-target lesions should be noted at Baseline and should be identified as "present (Non-CR/Non-PD)" or "absent (CR)" and (in rare cases) may be noted as "Unequivocal progression (PD)" at subsequent evaluations. In addition, if a lymph node lesion shrinks to a non-malignant size (short axis < 10 mm), this should be captured on the CRF as part of the assessment of non-target lesions.

4 RESPONSE CRITERIA

4.1 Criteria for Target Lesions

Definitions of the criteria used to determine objective tumour response for target lesions are provided below:

- Complete response (CR): Disappearance of all target lesions

  Any pathological lymph nodes must have reduction in short axis to < 10 mm.
- Partial response (PR): At least a 30% decrease in the sum of dimensions of all target lesions, taking as reference the Baseline sum of dimensions, in the absence of CR
- Progressive disease (PD): At least a 20% increase in the sum of dimensions of target lesions, taking as reference the smallest sum of dimensions on study (including Baseline)

In addition to the relative increase of 20%, the sum of dimensions must also demonstrate an absolute increase of ≥ 5 mm.

- Stable disease (SD): Neither sufficient shrinkage to qualify for CR or PR nor sufficient increase to qualify for PD

4.2 Criteria for Non-Target Lesions

Definitions of the criteria used to determine the tumour response for the group of non-target lesions are provided below. While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only qualitatively at the timepoints specified in the schedule of activities.

- CR: Disappearance of all non-target lesions and (if applicable) normalisation of tumour marker level

  All lymph nodes must be non-pathological in size (< 10 mm short axis).

- Non-CR/Non-PD: Persistence of one or more non-target lesions and/or (if applicable) maintenance of tumour marker level above the normal limits
- PD: Unequivocal progression of existing non-target lesions

4.3 Special Notes on Assessment of Progression of Non-Target Lesions

4.3.1 Patients with Measurable and Non-Measurable Disease

For patients with both measurable and non-measurable disease to achieve unequivocal progression on the basis of the non-target lesions, there must be an overall level of substantial worsening in non-target lesions in a magnitude that, even in the presence of SD or PR in target lesions, the overall tumour burden has increased sufficiently to merit discontinuation of therapy. A modest increase in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target lesions in the face of SD or PR in target lesions will therefore be extremely rare.

4.3.2 New Lesions

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal, that is, not attributable to differences in scanning technique, change in imaging modality, or findings thought to represent something other than tumour (for example, some "new" bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's Baseline lesions show PR or CR. For example, necrosis of a liver lesion may be reported on a CT scan report as a "new" cystic lesion, which it is not.

A lesion identified during the study in an anatomical location that was not scanned at Baseline is considered a new lesion and will indicate disease progression.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, progression should be declared using the date of the initial scan.

4.4 Criteria for Overall Response at a Single Timepoint

The table below provides a summary of the overall response status calculation at each response assessment timepoint for patients who have measurable disease at Baseline.

Criteria for Overall Response at a Single Timepoint: Patients with Target Lesions (with or without Non-Target Lesions)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not all evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

CR = complete response; NE = not evaluable; PD = progressive disease; PR = partial response; SD = stable disease.

4.5 Missing Assessments and Not-Evaluable Designation

When no imaging/measurement is done at all at a particular timepoint, the patient is not-evaluable at that timepoint. If measurements are made on only a subset of target lesions at a timepoint, usually the case is also considered not-evaluable at that timepoint, unless a convincing argument can be made that the contribution of the individual missing lesions would not change the assigned timepoint response. This would be most likely to happen in the case of PD. For example, if a patient had a Baseline sum of 50 mm with three measured lesions and during the study only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

4.6 Special Notes on Response Assessment

Patients with a global deterioration in health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as having "symptomatic deterioration." Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response; it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target lesions as shown in the above table.

For equivocal findings of progression (e.g., very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

APPENDIX C: ECOG

Grade	Description
0	Fully active; able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework or office work).
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about > 50% of waking hours.
3	Capable of only limited self-care; confined to a bed or chair > 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.
ECOG = Eastern Cooperative Oncology Group.	