Neoadjuvant AGS-003 Immunotherapy in Patients with Localized Kidney Cancer <pT2

Roswell Park Cancer Institute

Study Number: **I 250113**

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Manufacturer: **Argos Therapeutics, Inc.**

Argos Study Reference Number: **AGS-003-012**

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Study personnel outside of RPCI-CRS who will be housing essential documents or receiving, analyzing or storing biological samples

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SYNOPSIS

Title	**Neoadjuvant AGS-003 Immunotherapy in Patients with Localized Kidney Cancer <pT2**
RPCI Study No.	I 250113
RPCI Investigator	Dr.med.Thomas Schwaab, MD
Manufacturer	Argos Therapeutics, Inc. 4233 Technology Drive Durham, NC 27704-2173
Investigational Study Drug	AGS-003: A Suspension for Injection, consisting of cryopreserved *in vitro* transcribed (IVT)-RNA-electroporated and cultured autologous mature dendritic cells (DCs), formulated in autologous plasma, DMSO and dextrose for injection.
Objectives	**Primary:** To assess the immune-modulatory systemic and intratumoral effects of AGS-003as neoadjuvant treatment in patients with localized renal cell carcinoma. **Secondary:** To assess the feasibility that total tumor RNA processing-related activities meet specifications for AGS-003 manufacturing utilizing a core needle biopsy procedure for tumor harvesting prior to nephrectomy.
Study Design	This is a non-randomized single center pilot trial, treating patients who are eligible to undergo a partial or radical nephrectomy with the autologous AGS-003 immunotherapy in a neoadjuvant fashion prior to surgery. Peripheral (blood) and intratumoral parameters will be assessed.
Target Accrual & Study Duration	A maximum of 10 patients at RPCI will be enrolled. Study accrual is expected to take 4 years. Patients will participate in this study for approximately 6 months from the time of consent through final study visit.
Study Procedures	**Disease Evaluation:** Screening. **Adverse Events assessments:** Throughout the study. **Hematology and Chemistry:** Screening, Injection Week #1, Injection Week #3, Nephrectomy Visit, and End of Study. **Autoimmune Assessment**: Screening, Injection Week #1, Injection Week #3, Nephrectomy Visit, and End of Study. **Immunologic Analysis Blood Draw:** Injection Week #1, Nephrectomy Visit, and End of Study. **Pregnancy Test (urine) in females of childbearing potential:** Screening and End of Study. **Performance Status:** Screening, Injection Week #1, Injection Week #2, Injection Week #3, and End of Study. **Physical Examination:** Screening and End of Study**.** **Tumor biopsy for manufacture of AGS-003:** Screening. **Leukapheresis for manufacture of AGS-003:** Leukapheresis Visit (must be at least 48 hours between tumor biopsy and leukapheresis) **AGS-003 Dosing:** Once-weekly dosing beginning at Injection Week #1 for

	3 weeks.
Statistical Analysis	**Sample Size Determination:** A maximum of 10 patients will be enrolled in this study. The primary objective of this study is to assess immune-modulatory systemic and intratumoral effects of AGS-003 as neoadjuvant treatment in patients with localized renal cell carcinoma. Peripheral blood samples for immunological analysis will be collected before the first dose of AGS-003, after the 3rd dose of AGS-003, and at the end of the study. The time component will be modeled as a three-level classification factor. The full model for the effects of injection mode and time will be fit using Linear Mixed Model methods. The model will include a random patient effect and five fixed effects for time, and the interactions. The presence of any time effect will be assessed with full-reduced model Type 3 test. If the omnibus test is statistically significant at the $p < 0.05$ level, then three pairwise time-point comparisons will be conducted. **Safety Analysis:** Adverse event rates will be summarized in all patients who receive at least 1 or more doses of AGS-003. These rates will be described as the proportion of patients with the event, by grade, and supported with exact 95% confidence intervals. **Stopping Rule:** If during the trial 4 or more patients experience the undesirable event defined as unexpected bleeding or more difficult than usual perinephric dissection, accrual will be halted and the treatment will be deemed unsafe. **Correlative Data Analysis:** Peripheral blood immune cell populations will be analyzed using flow cytometry, ELISA, and ELISPOT assays. Tumor tissue will be processed and analyzed using flow cytometric and immunohistochemical methods.

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Patient Name: _____

Medical Record No.: _____

Title: I 250113: Neoadjuvant AGS-003 Immunotherapy in Patients with Localized Kidney Cancer <pT2

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for patient enrollment.**	**Date**
☐	☐	☐	1. Age ≥ 18 years of age.	
☐	☐	☐	2. Have localized non-metastatic RCC (< pT2, N0, MO) as per the AJCC 7th edition criteria[1]. Patients who have not had a biopsy must have a solid renal mass suggestive of RCC with confirmation of RCC at screening biopsy.	
☐	☐	☐	3. Must be surgical candidates as deemed fit by surgeon.	
☐	☐	☐	4. Patients of child-bearing potential must agree to use adequate contraceptive methods (e.g., hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform the treating physician immediately.	
☐	☐	☐	5. Willingness to undergo leukapheresis and biopsy procedures for the autologous components (i.e., peripheral blood mononuclear cells, plasma and fresh tumor specimen) required for manufacture of AGS-003 immunotherapy.	
☐	☐	☐	6. Patient or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.	

Investigator Signature: _____**Date:** _____

ROSWELL PARK CANCER INSTITUTE

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Patient Name: _____

Medical Record No.: _____

Title: I 250113: Neoadjuvant AGS-003 Immunotherapy in Patients with Localized Kidney Cancer <pT2

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for patient enrollment.**	**Date**
☐	☐	☐	1. Patients who have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier.	
☐	☐	☐	2. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure (e.g., shortness of breath, fatigue, orthopnea, paroxysmal nocturnal dyspnea), unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.	
☐	☐	☐	3. Radiation to primary tumor prior to enrollment in this study.	
☐	☐	☐	4. Pregnant or nursing female patients.	
☐	☐	☐	5. Unwilling or unable to follow protocol requirements.	
☐	☐	☐	6. Active autoimmune disease or condition requiring chronic immunosuppressive therapy (e.g., rheumatoid arthritis, systemic lupus erythematous, multiple sclerosis, organ transplant recipient, etc.). • NOTE: Abnormal laboratory values for autoimmunity markers in the absence of other signs/symptoms of autoimmune e disease are not exclusionary.	
☐	☐	☐	7. Known clinically significant infections, including human immunodeficiency virus (HIV) and active hepatitis B or C.	
☐	☐	☐	8. Any condition which in the Investigator's opinion deems the patient an unsuitable candidate to receive treatment (i.e., any significant medical illness or abnormal laboratory finding that would, in the investigator's judgment, increase the subject's risk by participating in this study).	
			9. Chronic use of systemic corticosteroids (i.e., ≥ 10 mg/day prednisone or equivalent).	
☐	☐	☐	10. Received an investigational agent within 30 days prior to enrollment.	

Patient meets all entry criteria: ☐ **Yes** ☐ **No**

Investigator Signature: _____ **Date:** _____

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APPENDICES

1 BACKGROUND

1.1 Renal Cell Carcinoma

Renal Cell Carcinoma (RCC) is the most lethal urologic cancer. Up to 30% of patients will present with metastatic disease. RCC is one of the few solid malignancies with continuously rising incidence over the past decade(2). Up to 40% of patients who undergo partial or radical nephrectomy for clinically localized RCC will recur as metastatic disease. High-dose Interleukin-2 (HD IL-2) is the only FDA-approved treatment with curative and long lasting results (3, 4). However, toxicities are high because HD IL-2 based immunotherapy is a non-specific immunostimulant leading to severe autoimmune reactions. There is a need for more tumor-specific immunotherapies to avoid these autoimmune toxicities in this highly immune-reactive cancer. Immunotherapy research has focused on identifying a more targeted approach by identifying and utilizing tumor-specific antigens.

1.2 AGS-003 Product Summary

Refer to Argos Therapeutics, Inc. AGS-003 Investigator's Brochure for more information on AGS-003, including Pharmacology, Immunologic Activity, Potency, Toxicology, and Safety.

Argos Therapeutics, Inc. (Argos) is an immunotherapy company with corporate headquarters in Durham, NC, and is the manufacturer of AGS-003. AGS-003 is an autologous active cellular immunotherapy designed to activate specific T cells that will assist in identifying and attacking unique antigens found on each subject's own tumor cells. AGS-003 utilizes autologous dendritic cells (DCs) co-electroporated with ribonucleic acids (RNAs) encoding autologous RCC antigens (derived from the patient's tumor biopsy specimen) and synthetically derived CD40L (CD40 is a glycoprotein expressed by DCs and DC maturation and T cell activation is significantly improved by CD40L stimulation.). The autologous precursor components for AGS-003 are biopsy-derived tumor specimen and leukocytes obtained via leukapheresis.

Tumor specimens collected from the subject during the biopsy procedure are placed in a formaldehyde-free, RNA preservative solution and shipped for next day delivery to Argos. In an aseptic processing area at Argos, tumor RNA is isolated from the specimen. Total cellular RNA is amplified via reverse transcriptase (RT) polymerase chain reaction (PCR) and the resulting complimentary deoxyribonucleic acid (cDNA), highly enriched for copies of messenger RNA (mRNA) from the original tumor specimen, is purified. The cDNA is used to generate and further amplify RNA using an *in vitro* transcribed (IVT*)* system. Thus, RNA encoding potentially all of the patient's tumor sample antigens is amplified for electroporation.

Leukocytes are obtained from subjects by leukapheresis using standard institutional procedures in accordance to Argos collection parameters, and are express shipped to the Argos facility. At the Argos facility, DC precursors (monocytes) are isolated from the leukocytes in the aseptic processing area by elutriation and are cultured to achieve DC maturation.

Post-maturation, DCs are electroporated with autologous amplified tumor and CD40L RNA. After RNA electroporation, the DCs are cultured to generate the final cellular preparation. This production method is known as the post-maturation electroporation (PME) -CD40L process. The resulting RNA-electroporated mature DCs are suspended in autologous plasma, 10% dextrose for

injection, and 10% dimethyl sulfoxide (DMSO), for cryopreservation, to comprise the finished product and frozen for storage until use.

All manufacturing and storage of the AGS-003 product is performed under current Good Manufacturing Practices (cGMP) by Argos. The final cGMP product is released for clinical use only if it passes all quality control testing, including tests for potency, sterility, and (absence of) endotoxin and mycoplasma.

Dendritic cells are antigen presenting cells (APCs) that can stimulate cell-mediated immunity through effects on both $CD4^+$ and $CD8^+$ T cells. The current hypothesis for the AGS-003 product is that the autologous RNA-electroporated DCs present tumor antigens to both memory and naive or resting T cells resulting in an expanded antigen-specific immune response.

It has been previously demonstrated that a typical adaptive immune response requires the integration of 3 signals: 1.) the major histocompatibility complex (MHC)/antigenic peptide interaction, 2.), the engagement of co-stimulatory molecules such as CD80 and CD86 and, 3.) the intercellular activity of IL-12 (5). AGS-003 incorporates all 3 of these signals in a single cellular product, enabling an adaptive immune response in RCC subjects who are otherwise immunocompromised (6).

In RCC, the normal immune surveillance function is thought to be compromised by the recruitment of regulatory T cells that suppress the anti-tumor response (7). When used as a single-agent, AGS-003 reversed tumor-induced immunosuppression in a majority of subjects tested (AGS-003-004). This was evident by the restoration of IFN-γ secretion by T cells isolated from subjects receiving AGS-003.

1.3 Pharmaceutical Properties of AGS-003

AGS-003 is an autologous dendritic cell immunotherapy. It consists of DCs co-electroporated with amplified autologous tumor RNA and CD40L RNA. The autologous tumor RNA is used to direct the immune response to the subject's tumor. The CD40L RNA is used to induce secretion of IL-12 leading to activation of effector memory cytotoxic T lymphocytes (CTLs). A strong correlation between IL-12 and the induction of effector memory CTLs has been demonstrated (8). Therefore, the potency of AGS-003 is in part determined by secretion of IL-12 from the cellular product (DCs after electroporation and culture). IL-12 plays a role in the induction of multi-functional effector memory CTLs and is considered a marker of immune cell differentiation.

Dendritic cell-based immunotherapeutics must induce robust CTLs capable of killing tumor *in vivo* if they are to be clinically effective. Argos has shown that AGS-003 generates high-avidity CTLs *in vitro* that lyse cells that express naturally processed and presented tumor antigen (Ag). Unlike cytokine cocktail-matured DCs, which induce predominantly non-proliferative effector memory $CD45RA^+$ CTLs, AGS-003 primes a novel subset of antigen-specific CTLs that can be expanded to large numbers upon sequential DC stimulation *in vitro*. Argos has defined these cells as REHA (rapidly expanding high avidity) CTLs based on: 1.) the maintenance of CD28 expression, 2.) production of high levels of IFN-γ and IL-2 in response to Ag and, 3.) the demonstration of high-avidity T cell receptors (TCRs) that exhibit strong cytolytic activity

toward limiting amounts of native Ag (9). These results suggest that AGS-003 DCs, consistent with their ability to express IL-12, are uniquely capable of delivering the complex array of signals needed to generate stable $CD28^{+}/CD45RA^{-}$ REHA CTLs, which, if generated *in vivo*, could be clinically beneficial for treatment of metastatic RCC. In support of this hypothesis, clinical immune monitoring results from the AGS-003-004 study showed that AGS-003 induces polyclonal CTL response to tumor antigens and that a subset of responding T cells secrete the cytokines IFN-γ and IL-2, suggesting an effector memory or central memory phenotype.

1.4 Clinical Studies with AGS-003

AGS-003 is the second-generation product based on the proprietary Arcelis™ technology for producing RNA-electroporated DCs for cancer immunotherapy. The first-generation product, MB-002, was virtually identical to AGS-003 in its autologous components, but did not include synthetically derived CD40L RNA nor did it utilize the sequential DC maturation process. The purpose of adding this CD40L RNA is to provide the CD40/CD40L ligation signal required by the DCs to induce IL-12 secretion (10). IL-12 has been demonstrated to be linked to the functionality of the DC (8), and assessment of IL-12 secreted from the DC product serves as a potency marker for the release of the AGS-003 product.

To date, in over 6 years of clinical experience, a total of 63 newly diagnosed, unfavorable risk subjects with metastatic RCC received a total of over 500 doses of either AGS-003 or MB-002 in Phase I/II and Phase II studies, with dosing of multiple years' duration for some subjects. The first 2 clinical trials, MB-002-003 and AGS-003-004, were open-label, single-arm studies of MB-002 and AGS-003, respectively, as first-line monotherapy in subjects with advanced RCC. The third trial, AGS-003-006, was a single-arm Phase II study that used a combination therapy of AGS-003 plus sunitinib in previously untreated subjects with advanced RCC. Both safety and efficacy results from these studies have been encouraging.

A Phase III trial, AGS-003-007, is ongoing in patients with advanced RCC. This is a randomized trial that will compare the safety and efficacy of AGS-003 plus standard treatment versus standard treatment alone. For both study arms, standard treatment will begin with sunitinib (administered per current product labeling, and initiating with 50 mg/day for 4 weeks followed by a 2 week drug-free period each cycle, unless indicated otherwise by current labeling).

1.5 Clinical Immuno-dynamics of AGS-003 Monotherapy

In all clinical studies of AGS-003 and the predecessor product MB-002, blood samples collected from subjects at defined points on study have been assessed for monitoring of immune function. In Study MB-002-003, 8 of 12 subjects that were evaluable for immune monitoring recovered some measure of immunocompetency after treatment and their T cells were able to mount an IL-2 immune response to more than one stimulus. However, immune response monitoring indicated this first generation product (MB-002) did not fully overcome the apparent immune deficiency in RCC subjects (notably, although the IL-2 response was rescued, no restoration of IFN-γ responses was observed).

For study AGS-003-004, as a measure of general immune system health, the total response to DC re-stimulation was analyzed at 4 time points: two before treatment and two post-treatment.

The majority of study subjects exhibited increased immune responsiveness to a variety of immunogens after AGS-003 therapy, which may indicate general immune reconstitution since some test immunogens were not part of the AGS-003 RNA payload. Overall, 12 of 16 subjects (75%) had a response over post-treatment to at least one of the tumor antigens [refer to Investigator's Brochure (IB)], suggesting that RCC subject T cells can mount a polyclonal response to multiple antigens associated with their own tumors when antigen is encoded as RNA that is electroporated into DCs matured with the PME-CD40L process. A subset of the T cell response was associated with T cells bearing both IFN-γ and IL-2. This profile is associated with T cells exhibiting "effector memory" and/or "central memory" activities (9).The hallmark of effective immune therapy is the induction of "immunological memory".

1.6 AGS-003 Safety

Previous clinical studies have demonstrated that single-agent AGS-003 and its predecessor product MB-002 are safe and well-tolerated. With more than 500 doses delivered across these studies, most drug-related adverse events (AEs) have been mild to moderate injection site reactions, with no reports of any drug-related AEs with Grade 3 or Grade 4 toxicity, and no drug-related serious adverse events (SAEs). No subjects in any studies of AGS-003 or MB-002 have developed autoimmune disease, and periodic assessments of autoimmunity markers in these studies have yielded no clinically significant findings. Of note, in all RCC studies each subject received AGS-003 manufactured from the subject's tumor (primary or metastatic RCC site), yet no subjects developed autoimmune reactions against their remaining (healthy) kidney.

2 RATIONALE

Dendritic cells (DC) are the most potent cells in initiating antigen-specific T cell responses. In particular, they are key players in the initiation of so-called cross presentation. Cross presentation is crucial for the induction of both CD8 cytotoxic T cells and CD4 memory T cells to initiate a memory immune response. The clinical and immunologic potential of an autologous DC immunotherapy was demonstrated in a recent Phase II trial (11). Patients with metastatic kidney cancer received an autologous, tumor-lysate pulsed DC vaccine and showed a median progression free survival (PFS) of 8 months. The median OS has not been reached. Nine objective responses were noted (50%; 95% CI 22%-66%), which included complete responses in 2 patients (19 and 24 months) and 7 partial responses (3-9 months). This trial demonstrated that an autologous DC vaccine can be prepared in a consistent, high-quality fashion (11). Autologous tumor lysate provides a large number of target antigens, but it requires a large volume of tumor tissue. Of particular importance, tumor specimen obtained from reduced quantity of starting tumor tissue, such as from a core needle biopsy procedure, should routinely provide sufficient total tumor RNA to initiate the RNA amplification process, making available ample source material for dendritic cell transfection. AGS-003 combines the most up-to-date DC vaccine technology with the option of targeting a plethora of autologous, tumor-specific target antigens. The data on the clinical efficacy of AGS-003 is becoming increasingly apparent, however, the direct anti-tumor mechanism of action is as of yet undefined. The proposed research study will analyze this mechanism of action. In addition, this neoadjuvant concept of preoperative AGS-003 immunotherapy will lead to better understanding of the role of neoadjuvant immunotherapy

as a means to potentially facilitate tumor resections and may even provide the option of "downsizing" a kidney tumor and change the surgical approach from radical to partial nephrectomy, thus providing significant preservation of precious renal function. If this proposed study demonstrates the expected systemic and intratumoral immune responses, it will lead to the design of a larger Phase II study.

3 OBJECTIVES

3.1 Primary Objective

- To assess the immune-modulatory systemic and intratumoral effects of AGS-003 as neoadjuvant treatment in patients with localized renal cell carcinoma.

3.2 Secondary Objective

- To assess the feasibility that total tumor RNA processing-related activities meet specifications for AGS-003 manufacturing utilizing a core needle biopsy procedure for tumor harvesting prior to nephrectomy (see Argos "Summary of Experience with RNA Amplification from Various Types of Primary Tumors").

4 METHODOLOGY

4.1 Study Design

This is a non-randomized, single center Pilot study treating patients with non-metastatic RCC with the autologous dendritic cell AGS-003 as a neoadjuvant treatment prior to extirpative radical or partial nephrectomy.

Eligible patients will be administered the AGS-003 as a single, fixed dose of 1.2×10^7 DCs, delivered as an intradermal injection (0.6 mL per dose delivered as three separate 0.2 mL injections per full dose) once weekly for a total of 3 doses beginning at Injection Week #1, followed by a partial or radical nephrectomy at the Nephrectomy Visit. Blood will be taken for immunological analysis at the time of the first dose, after the 3rd dose, and at End of Study.

All patients will sign an informed consent prior to study related tests. All patients will meet the inclusion and exclusion criteria. Patients will be treated on an outpatient basis. Kidney surgery will require inpatient admission postoperatively.

4.2 Target Accrual and Study Duration

A maximum of 10 patients at RPCI will be enrolled. Study accrual is expected to take 4 years. Patients will participate in this study for approximately 6 months from the time of consent through the final study visit.

5 PATIENT SELECTION

5.1 Inclusion Criteria

To be included in this study, patients must meet the following criteria:

1. Age ≥ 18 years of age.
2. Have localized non-metastatic RCC (<pT2, NO, MO), as per the AJCC 7th edition criteria(1). Patients who have not had a biopsy must have a solid renal mass suggestive of RCC with confirmation of RCC at screening biopsy.
3. Must be surgical candidates as deemed fit by surgeon.
4. Patients of child-bearing potential must agree to use adequate contraceptive methods (e.g., hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform the treating physician immediately.
5. Willingness to undergo leukapheresis and biopsy procedures for the autologous components (peripheral blood mononuclear cells, plasma and fresh tumor specimen) required for manufacture of AGS-003.
6. Patient or legal representative must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.

5.2 Exclusion Criteria

Patients will be excluded from this study for the following:

1. Patients who have had chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier.
2. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure (e.g., shortness of breath, fatigue, orthopnea, paroxysmal nocturnal dyspnea), unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
3. Radiation to primary tumor prior to enrollment in this study.
4. Pregnant or nursing female patients.
5. Unwilling or unable to follow protocol requirements.
6. Active autoimmune disease or condition requiring chronic immunosuppressive therapy (e.g., rheumatoid arthritis, systemic lupus erythematous, multiple sclerosis, organ transplant recipient, etc.).

- NOTE: Abnormal laboratory values for autoimmunity markers in the absence of other signs/symptoms of autoimmune disease are not exclusionary.

7. Known clinically significant infections, including human immunodeficiency virus (HIV) and active hepatitis B or C
8. Any condition which in the Investigator's opinion deems the patient an unsuitable candidate to receive treatment (i.e., any significant medical illness or abnormal laboratory finding that would, in the investigator's judgment, increase the subject's risk by participating in this study).
9. Chronic use of systemic corticosteroids (i.e., ≥ 10 mg/day prednisone or equivalent).
10. Received an investigational agent within 30 days prior to enrollment.

5.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this study.

6 TREATMENT PLAN

6.1 Study Procedures for Manufacture of AGS-003

AGS-003 will be manufactured using autologous specimens obtained from the procedures described below. As described below, tumor specimens obtained by percutaneous biopsy will be collected for all study subjects at Screening. Also, as described below, peripheral blood mononuclear cells (PBMCs) and plasma will be collected by leukapheresis.

The autologous starting materials for the AGS-003 product are peripheral blood mononuclear cells (PBMCs) obtained by leukapheresis, RCC tissue obtained from a core needle biopsy, and plasma obtained with the PBMCs during leukapheresis. These components are then shipped directly to Argos for manufacturing at a centralized facility under current Good Manufacturing Practices (cGMP). Monocytes are isolated from the PBMCs and are differentiated *in vitro* to generate dendritic cells (DCs), while the tumor biopsy specimen is used to isolate and amplify ribonucleic acid (RNA) that encodes autologous RCC antigens. AGS-003 is produced by electroporating the DCs with the tumor-derived amplified RNA, as well as with synthetically derived RNA that encodes the protein cluster of differentiated 40 ligand (CD40L).

If there is a failure to isolate/amplify the RNA from the tumor biopsy specimen, the patient will be removed from the study and will proceed with standard of care treatment (i.e. partial or radical nephrectomy).

Refer to the AGS-003 Dose Management Manual for detailed instructions.

6.1.1 Tumor Specimen for AGS-003

Tumor specimens will be used for the manufacturing of AGS 003. Tumor tissue from a core needle biopsy will be obtained at the study site from each study participant at Screening, following standard institutional procedures. The tumor specimen containers will be shipped

directly to Argos. Further instructions for tumor specimen collection, shipping, and handling are provided in the Argos Guideline for Core Biopsy Tumor Collection for RNA Preservation.

Tumor specimens may be stored for up to 2 years after the last subject's final study visit or per local regulatory requirements.

6.1.2 Leukapheresis

The AGS-003 manufacturing process requires leukapheresis to collect autologous leukocytes. Study patients will be referred to the American Red Cross in Buffalo (3601 Union Rd, Cheektowaga, NY 14225). Even though leukapheresis occurs prior to administration of investigational product, RPCI will report to the FDA severe (Grade 3, CTCAE) or life-threatening (Grade 4, CTCAE) events associated with the leukapheresis.

The leukapheresis should not be performed less than 48 hours after the tumor biopsy procedure (Section 6.1.1).

In most cases, only a single leukapheresis will be required to support product manufacture. However in some cases, cellular collection or manufacturing with the initial product is unsuccessful, and a second or third leukapheresis may be required. If additional leukapheresis (es) is required, standard treatment should still be initiated as clinically indicated.

6.2 Dosing and Administration

AGS-003 will be manufactured and supplied in a single dose vial for each patient enrolled in the study, and will consist of autologous RNA-electroporated, mature DCs, 1.2×10^7 DCs per dose formulated in autologous plasma, 10% dimethyl sulfoxide (DMSO), and 10% Dextrose for Injection (50% w/v).

AGS-003 study drug stocks are not stored long term at clinical sites. Instead, each individual dose will be shipped to RPCI 7 North Pharmacy according to a schedule mutually agreed upon by RPCI and Argos, under controlled conditions and in accordance will FDA approved guidelines for INDs. It will remain on 7-North unopened until administration.

AGS-003 is for autologous use ONLY. Use is absolutely limited to the individual subject who is the autologous donor of the tumor, PBMCs and plasma used in the individual product lot identified by the SID (subject identification number).

All injections will be administered by a protocol-trained health care provider.

The target dose of AGS-003 (1.2×10^7 DCs) is delivered in three intradermal injections of 0.2 mL each (0.6 mL total volume) at Injection Weeks #1, #2, and #3 (every 7 days, ± 2 days), followed by a partial or radical nephrectomy at the Nephrectomy Visit. Injections will be administered, with the location of injection sites documented by the Principal Investigator or protocol-trained medical personnel.

6.2.1 Intradermal Injection

AGS-003 is administered via standard intradermal injection techniques: the entire dose (distributed in 3 syringes) is targeted to a single lymph node basin. The dose is delivered in three

separate injection sites (approximately 5 cm from each other) in the axillary lymph node basin (or inguinal if needed), delivering 0.2 mL of study product at each site for 0.6 mL total, with subsequent doses alternating between the right and left sides. If axillary injections are not feasible (e.g., because of lymphedema or extensive Injection Site Reactions from preceding administrations), inguinal injections are allowed, alternating between the right and left groin.

The treatment will be administered on an outpatient basis on 7-North of the RPCI main hospital. The Principal Investigator will demonstrate the injection technique to the nursing staff on 7-North and, subsequent injections will be administered by the protocol-trained nursing staff.

Following the injection, patients will remain on 7-North and will be monitored as outpatients for 1 hour after AGS-003 injection. Monitoring will consist of vital sign measurements (temperature, heart rate, respiration rate, and BP) and local/systemic reactions to the injection. Patients will be monitored for side effects including inflammation and swelling in the draining lymph nodes. This monitoring will consist of clinical assessment of pain, tenderness, warmth and swelling. In the absence of adverse events, patients will be discharged 1 hour after injection from the outpatient clinic.

6.3 Dose Modifications

Dose modification of AGS-003 is not permitted. AGS-003 should be administered per protocol regardless of changes in standard treatment.

6.4 General Concomitant Medication and Supportive Care

6.4.1 Permitted Medications

Permitted medications include, but are not limited to, the following:

- Standard vaccinations (e.g., influenza vaccine)
- Intermittent use of antibiotics for sporadic infections as indicated
- Bisphosphonates
- Denosumab
- Granulocyte colony-stimulating factor (G-CSF)
- Erythropoietin stimulating agents
- Transfusions of blood or blood products
- Warfarin or low molecular weight heparin (LMWH)

Corticosteroid use is permitted ONLY as defined below:

- $\leq$ 10 mg/day prednisone or equivalent (e.g. $\leq$ 50 mg/day cortisone) and replacement steroids are permitted

- Use of inhaled corticosteroids (e.g., for chronic obstructive pulmonary disease [COPD]) or ophthalmic steroids are permitted, but, if possible, should not be taken on the date of AGS-003 dosing
- Intermittent topical use of corticosteroids (i.e., creams) to small areas of the skin, after consultation with the investigator (except that ISRs should not be treated with corticosteroids, as noted below)

NOTE: Mild and moderate ISRs are expected and should resolve without sequelae. Injection site reactions should not be treated with any form of corticosteroids or antihistamines. If desired, non-steroidal anti-inflammatory topical ointments or drugs (NSAIDS) such as ibuprofen may be used to alleviate symptoms.

6.4.2 Prohibited Medications

The following medications are prohibited:

- Concomitant immunosuppressive or immunomodulatory agents
- Use of cytokines (including products containing interferons or interleukins), or granulocyte macrophage colony stimulating factor (GM-CSF). NOTE: G-CSF is permitted (**Section 6.4.1**)
- Chronic use of systemic corticosteroids, topical corticosteroids that are applied to large areas of the skin (exceeding the cumulative area of the palm of the subject's hand), or any corticosteroids or antihistamines used on or near the injection sites, except as noted above under "Permitted Medications"

6.4.3 Drug Interactions

There are no drugs that are known to directly interact with AGS-003. However, sorafenib, specified cytotoxic drugs, and chronic immunosuppressive therapies may negatively impact the mechanism of action of AGS-003, and are prohibited concomitant medications.

6.5 Treatment Discontinuation

All patients who discontinue due to an AE must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of the AE will be reported in the patient's medical records and the appropriate eCRF.

Reasons for treatment discontinuation should be classified as follows:

- Death
- Progressive disease
- Treatment-related toxicity
- Toxicity unrelated to treatment
- Investigator judgment

- The Investigator may withdraw a patient if, in his/her judgment, it is in the patient's best interest to do so
- Noncompliance
- Patient voluntary withdrawal
- A patient may withdraw from the study at any time, for any reason. If a patient discontinues treatment, an attempt should be made to obtain information regarding the reason for withdrawal.
- Sponsor decision
- Development of active autoimmune disease or other condition requiring chronic immunosuppressive therapy (such as rheumatoid arthritis, systemic lupus erythematous, multiple sclerosis, organ transplant recipient, etc.)
- If, while on study, a patient shows any evidence of tumor progression independent of any SAE, study treatment will be discontinued, the patient's RCC will be restaged, and the study investigator will discuss possible treatment options (including definitive therapy) with the patient.
- If the study product is delayed > 2 weeks, the patient will discontinue the study and be allowed to proceed directly onto surgery and, the patient would be replaced in the study.

7 INVESTIGATIONAL PRODUCT

7.1 Active Substance and Source

AGS-003 (IVT RNA-electroporated and cultured autologous mature DCs) is a personalized immunotherapeutic product whose autologous components are DCs (from a leukapheresis specimen) and RNA (from a tumor specimen). The tumor specimens and leukocytes are collected at the study site and shipped to Argos under controlled conditions in accordance with FDA-approved guidelines. AGS-003 is produced at Argos according to current Good Manufacturing Practice (cGMP). RNA that encodes autologous tumor antigens is isolated from the disease specimen and amplified. Monocytes are isolated from the leukapheresis collection and differentiated into DCs. The DCs are then co-electroporated with amplified tumor RNA as well as with RNA that encodes synthetically derived CD40L.

Refer to the AGS-003 IB for additional manufacturing information.

7.2 Risks/Benefits of AGS-003

Previous clinical studies have demonstrated that single-agent AGS-003 and its predecessor product MB-002 are safe and well-tolerated. Most drug-related AEs have been mild to moderate injection site reactions, with no reports of any drug-related AEs with Grade 3 or Grade 4 toxicity, and no drug-related serious adverse events (SAEs). No subjects in any studies of AGS-003 or MB-002 have developed autoimmune disease, and periodic assessments of autoimmunity markers in these studies have yielded no clinically significant findings. Of note, in all RCC

studies each subject received AGS-003 manufactured from the subject's renal tumor, yet no subjects developed autoimmune reactions against their remaining (healthy) kidney.

According to the literature, serious reactions to DC products are rare. Theoretical risks include allergic reactions such as hives or rash, and development of a systemic autoimmune response. Other possible side effects include headache, nausea, and vomiting. Systemic AEs have included influenza-like symptoms (e.g., fever), diarrhea, and fatigue.

Because AGS-003 is still in clinical development, it is unknown whether subjects who receive it will experience an improvement of their disease.

7.3 Packaging and Labeling

AGS-003 is packaged and labeled at the Argos facility. Each lot is manufactured for a specific subject, therefore; the product labeling will include a unique, 8 digit subject-specific identification code (SID). Refer to AGS-003 Autologous Dendritic Cell Therapy Dose Manual for additional information.

7.4 Drug Shipment

7.4.1 AGS-003 CryoShipper System

The AGS-003 dose will be transported in a liquid nitrogen CryoShipper system under controlled conditions at temperatures ≤ -150°C. The dry shipper system includes a "cryochamber" which is a small Dewar that has been charged with liquid nitrogen and that holds the dose in the vapor phase of this liquid nitrogen. The cryochamber in its shipping container must be stored UPRIGHT, in a secure location with access limited to study personnel only.

Each CryoShipper will also have a label on the exterior with an expiration date for the CryoShipper. The CryoShipper label contains the following information:

- Argos drug code (i.e. AGS-003)
- Subject Identification (SID) (i.e. PST00000)

Refer to AGS-003 Autologous Dendritic Cell Therapy Dose Manual for additional information.

7.4.2 Study Drug Shipments from Argos

Individual doses of Study Drug for injection will be shipped separately (to RPCI 7 North Pharmacy) for each scheduled administration. The site coordinator and Argos will coordinate to schedule shipment of each dose.

As soon as the CryoShipper is received, the designated staff must verify that the tamper-proof tape is intact and that the liquid nitrogen shipping container (CryoShipper) is not damaged. Remove the tamper proof tape, undo the latches and open the lid, then retrieve the shipping documents.

Refer to AGS-003 Autologous Dendritic Cell Therapy Dose Manual for additional information.

7.5 Dose Preparation and Administration

A detailed description and outline is provided in the AGS-003 Autologous Dendritic Cell Therapy Dose Manual.

AGS-003 is a biological product which contains live cellular material. AGS-003 is prepared under aseptic conditions and is formulated for administration as supplied, using aseptic injection techniques.

Each dose of AGS-003 will be provided as a single-dose vial for injection. The product is supplied frozen and must be thawed immediately prior to use.

Each dose of AGS-003 (0.6 mL total volume) will be delivered in three intradermal (i.d.) injections of 0.2 mL each, using the intradermal syringes provided with the dose shipment. Dose modifications are not permitted.

All injections should be administered within 30 minutes of vial removal from the cryogenic chamber (liquid nitrogen Dewar) to maximize cell viability. If more than 30 minutes has elapsed from the time the cryogenic chamber has been opened and single dose vial removed, administer the dose as soon as possible and notify Argos immediately.

Axillary or inguinal sites must be used for injections, with the entire dose (distributed in 3 syringes) targeted to a single lymph node basin. Axillary injection sites are preferred. Right and left axillary injections must be made in the lateral aspect of the chest wall as near to the shoulder as practical.

If axillary injections are not feasible (e.g. because of lymphedema or extensive Injection Site Reactions from preceding administrations), inguinal injections are allowed, alternating between the right and left groin. Inguinal injections must be made to the anteromedial aspect of the limb as close to the inner groin as possible.

All three injections comprising the single AGS-003 dose should be targeted to a single lymph node area. AGS-003 is administered to a lymph node basin to deliver dendritic cells as close to the lymph areas as possible. However, do not attempt to directly inject the actual lymph nodes.

In final preparation for dose administration, TWO staff personnel must compare the subject identification (SID) on the syringe labels to the SID listed in the subject's medical chart, and verify that the correct medical chart is present for the subject. Document this final dose verification in the Investigational Product Accountability Log.

Administer the product via standard intradermal injection techniques; the entire dose (distributed in 3 syringes) is targeted to a single lymph node basin. The dose is delivered in three separate injection sites in the axillary lymph node basin (or inguinal if needed), delivering 0.2 mL of study product at each site for 0.6 mL total. The 3 injection sites must be approximately 5 cm from each other, and subsequent doses will alternate between the right and left sides. Injections must be administered by a protocol-trained health care provider.

7.5.1 Injection Site Reactions

Mild and moderate injection site reactions are expected and should resolve without sequelae. Injection site reactions should not be treated with any form of corticosteroids or antihistamines. If desired, non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen may be used to alleviate symptoms. Should a more serious or systemic immune reaction (e.g., upper extremity, facial flushing) occur, standard treatment with antihistamines and other medications would be permitted to manage the reaction.

7.6 Handling and Disposal

AGS-003 is a biological product which contains live cellular material. AGS-003 is prepared under aseptic conditions and is ready for administration as supplied, using aseptic injection techniques. Since each dose of AGS-003 has been manufactured for use by a specific subject, verification procedures must be followed. The AGS-003 shipper must remain unopened at the clinical trial site until the subject is present and, both the subject and site staff are ready for dose administration.

The Investigator or designee will be responsible for dispensing and accounting for all investigational drug provided by Argos, exercising accepted medical and pharmaceutical practices. Study drugs must be handled as infectious agents and appropriate precautions taken per the institution's environmentally safe handling procedures. All investigational drugs will be dispensed in accordance with the Investigator's prescription or written order.

Additionally, for each study subject/dose, the site is instructed to record the time of vial removal from the shipper, the time of dose injection, the volume of dose administered, whether leak-back was observed from any injection sites, and a study drug disposition log that records the final disposition of each dose.

After dosing, any unused product must be destroyed. The clean-up and disposal of spilled, wasted, or unused medication and of used syringes must be documented appropriately in accordance with applicable federal regulations, Good Clinical Practice (GCP) procedures, and the procedures for handling biohazardous substances. Discard the empty vial and used syringes as you would any biohazardous material per institutional policies. Partially used vials of the study drug will not be re-used for other patients.

8 STUDY PROCEDURES

8.1 Patient Registration

Eligibility of each patient will be established prior to enrollment.

Informed consent *must* be completed prior to receiving any study-related procedures.

8.2 Screening

The following evaluations will be performed within 4 weeks prior to tumor biopsy:

- Medical history (including all prior anti-tumoral therapy related to RCC)

- Physical examination, including vital signs (i.e., temperature, heart rate, respiratory rate, blood pressure), body weight, and height
- Hematology:
  - (i.e., complete blood count (CBC) with automated differentials): WBC, RBC, HGB, HCT, platelet, MCV, MCH, MCHC, %neutrophils, absolute neutrophils, ,%monocytes, absolute monocytes, %eosinophils, absolute eosinophils, %basophils, absolute basophils, %lymphocyte total, absolute lymphocyte total, platelet confirmation (as clinically indicated), and differential confirmation (as clinically indicated).
- Chemistry:
  - Complete Metabolic Panel (i.e., CMP: chloride, CO_2, potassium, sodium, BUN, glucose, calcium, creatinine, total protein, albumin, bilirubin total, alkaline phosphatase, AST, ALT, osmol (calc), anion gap, B/C ratio, A/G ratio) along with gamma-glutamyl transferase (GGT), lactose dehydrogenase (LDH), phosphorus, magnesium, uric acid, thyroid stimulating hormone (TSH), c-reactive protein (CRP), and corrected calcium.
- Autoimmunity Assessment:
  - Rheumatoid factor (RF)
  - Anti-thyroglobulin antibody (TG)
  - Anti-double stranded DNA antibody (dsDNA antibody)
  - Anti-nuclear antibody (ANA)
- ECOG Performance Status (see **Appendix A**)
- Tumor assessment, including CT scan of the chest, abdomen, and pelvis
- Concomitant Medications List any ongoing medications with an onset within 1 week of tumor biopsy
- Pregnancy test (urine) in females of childbearing potential

Tumor biopsy for AGS-003 in accordance with Argos requirements will also be performed at Screening.

8.3 Leukapheresis Visit (must occur within 3 weeks after biopsy)

Leukapheresis: Leukapheresis for AGS-003 manufacturing should be performed 3 weeks after tumor biopsy. Scheduling of leukaphapheresis will be determined by American Red Cross availability. Patient may need second leukapheresis based on success of first one. All efforts will be made to ensure that second leukapheresis will be within the 3 week timeframe but will not be considered a protocol deviation if it falls out of this window.

8.4 AGS-003 Treatment Phase

The manufacturing of the vaccine can take 6-12 weeks. Due to the unexpected nature of the timeline of manufacturing, once the vaccine has been successfully created and ready to be shipped to RPCI, the study coordinator will work with Argos and all site staff to ensure that scheduling for proper administration and safety testing is performed. Injections should be given 7 days apart. Shipments are sent from Argos one dose at a time. Shipment delays and other unexpected events do occur therefore a dosing window of +/- 2 days will be allowed for each dose.

8.4.1 Injection Week #1

- Vital Signs, weight
- ECOG Performance Status
- Concomitant medications
- Adverse events
- Hematology, Chemistry, Autoimmune and Immunologic assessment prior to dosing
- Administer AGS-003, 1st dose

8.4.2 Injection Week #2

- Vital Signs, weight
- ECOG Performance Status
- Concomitant medications
- Adverse events
- Administer AGS-003 2nd dose

8.4.3 Injection Week #3

- Vital Signs, weight
- ECOG Performance Status
- Concomitant medications
- Adverse events
- Hematology, Chemistry, and Autoimmune assessment prior to dosing
- Administer AGS-003 3rd dose

8.4.4 Nephrectomy Visit

Partial or radical nephrectomy will be performed at this visit.

The following assessments are also performed at this time. If there is a pre-operative visit, the assessments may be performed as part of that visit instead. Otherwise, assessments are to be performed on day of scheduled nephrectomy, prior to surgery.

- Concomitant medications
- Adverse events
- Blood draw for Immunologic assessment
- Blood draw for Hematology, Chemistry and Autoimmunity assessment
- Tissue sample from nephrectomy.

8.5 End of Study Visit/ Follow-Up

The end of study visit is conducted approximately one month following nephrectomy, or any time the subject discontinues the study. The following assessments will be performed:

- Physical examination including vital signs and weight
- Hematology: Complete Blood Count with automated differential (CBC/DIFF)
- Chemistry: Complete Metabolic Panel (P22 COMP)
- Blood draw for Immunological analysis
- Blood draw for Autoimmunity assessment
- Pregnancy test (urine) in females of childbearing potential
- ECOG Performance Status (see **Appendix A**)
- Concomitant medication
- Adverse events (surgical AEs only if clinically significant)

8.6 Schedule of Procedures and Observations

The schedule of procedures and observations for this study is summarized in **Table 1**.

Table 1. Schedule of Procedures and Observations

	Screening[1]	Leukapheresis Visit	Injection Week #1	Injection Week #2	Injection Week #3	Nephrectomy Visit	End of Study/ Follow -Up[3]
Medical History	X						
Physical Examination	X						X
Vital Signs & ECOG[13]	X		X	X	X		X
Prior/Concomitant Medications	X[4]		X	X	X	X	X
Adverse Events			X	X	X	X	X
Chemistry[5]	X		X		X	X	X
Hematology [6]	X		X		X	X	X
Autoimmunity Assessment[7]	X		X		X	X	X
Pregnancy Test (urine)[8]	X						X
Tumor Assessment [9]	X						
Tumor Biopsy for AGS-003	X						
Leukapheresis for AGS-003[10]		X					
AGS-003 Dose[11]			1st	2nd	3rd		
Blood Sampling for Immunologic Assessment [12]			X			X	X
Nephrectomy						X	
Tissue from Nephrectomy						X	

Notes for Table 1

1. Performed within 4 weeks prior to Leukapheresis Visit.
2. If there is a pre-operative visit, Nephrectomy Visit assessments may be performed as part of that visit instead. Otherwise, assessments, including immunologic, are to be performed on day of nephrectomy prior to surgery.
3. Performed at post op visit following nephrectomy, or if subject discontinues or is withdrawn from the study.
4. Medications ongoing within 1 week prior to tumor biopsy.
5. **Chemistry**: Complete Metabolic Panel (i.e., CMP: chloride, CO_2, potassium, sodium, BUN, glucose, calcium, creatinine, total protein, albumin, bilirubin total, alkaline phosphatase, AST, ALT, osmol (calc), anion gap, B/C ratio, A/G ratio) along with gamma-glutamyl transferase (GGT), lactose dehydrogenase (LDH), phosphorus, magnesium, uric acid, thyroid stimulating hormone (TSH), c-reactive protein (CRP), and corrected calcium. NOTE: On Injection Weeks #1 and #3 assessments should be taken prior to dosing. At Nephrectomy Visit the assessments can be taken either at a pre-operative visit or on the day of nephrectomy prior to surgery.
6. **Hematology**:[(i.e., complete blood count (CBC) with automated differentials): WBC, RBC, HGB, HCT, platelet, MCV, MCH, MCHC, %neutrophils, absolute neutrophils, %monocytes, absolute monocytes, % eosinophils, absolute eosinophils, %basophils, absolute basophils,), %lymphocyte total, absolute lymphocyte total, platelet confirmation as clinically indicated. Grade 4 neutropenia should be monitored according to institutional guidelines. NOTE: On Injection Weeks #1 and #3 assessments should be taken prior to dosing. At Nephrectomy Visit the assessments can be taken either at a pre-operative visit or on the day of nephrectomy prior to surgery.
7. On Injection Weeks #1 and #3 assessment should be taken prior to dosing. At Nephrectomy Visit the assessment can be taken either at a pre-operative visit or on the day of nephrectomy prior to surgery: Rheumatoid Factor (RF), Anti-thyroglobulin (TG), Anti-double stranded DNA antibody (dsDNA antibody), Anti-nuclear antibody (ANA).
8. Only applicable to females of child-bearing potential.
9. To include CT scan of the chest, abdomen, and pelvis.
10. American Red Cross (3601 Union Rd, Cheektowaga, NY 14225) in accordance with Argos requirements. Should not be performed less than 48 hours after tumor biopsy procedure.
11. Perform any required Chemistry, Hematology, Autoimmune and Immunologic assessment blood draws **before** AGS-003 dosing. Observe possible Injection Site Reactions for 1 hour post injection. Assess Vital signs (temperature, heart rate, respiration rate, BP). The target dose of AGS-003 (1.2×10^7 DCs) is delivered in three intradermal injections of 0.2 mL each (0.6 mL total volume) at Injection Weeks #1, #2, and #3 (every 7 days, ± 2 days).
12. For *Argos Immune Monitoring*: Six, 10 mL green-top heparinized collection tubes, (tubes provided in kit). Refer to **Section 9.1.1**. For *Correlative Studies Samples*: Four, 10 mL green-top heparinized collection tubes: **Refer to 9.1.2**.

13. Screening vital signs: temperature, heart rate, respiration rate, BP, height and weight. Dose days and End of Study: all but height.

9 BIOLOGICAL SPECIMEN MANAGEMENT

9.1 Blood Sample Collection and Processing for Immunologic Analysis

9.1.1 Samples for Argos Immune Monitoring

Samples for immunologic analysis (six 10 mL green-top heparinized collection tubes) will be collected in the Phlebotomy Laboratory prior to the initial AGS-003 dose, after the 3rd dose, and at the final study visit. Samples will be sent to Laboratory Medicine for same day shipment to Argos Therapeutics, Inc. (Refer to Argos Immune Monitoring Sampling Kit Instructions and Immune Monitoring Sample Worksheet.) Immune monitoring samples are sent to the following address on the Day of Collection (Monday through Thursday):

Argos Therapeutics, Inc.

4233 Technology Drive

Durham, NC 27704-2173

immunomonitoring@argostherapeutics.com

9.1.2 Samples for Correlative Studies

Samples for the study correlative analyses (four 10 mL green-top heparinized collection tubes) will be collected in the Phlebotomy Laboratory at the same times as those for Immune Monitoring described above (prior to the initial AGS-003 dose, after the 3rd dose, and at the final study visit) and sent to the Hematological Procurement Facility.

Plasma and PBMC will be separated from the total blood within 30 minutes following the extraction. The screw cap polypropylene cryogenic tube will be labeled with the patient's MR number, patient's initials, patient's study number, clinical study number, protocol time point, dose, and protocol day. The label for each patient's sample will be supplied by RPCI. The samples will immediately be frozen at -70ºC or below until analyzed and the PBMCs will be stored in liquid nitrogen. The samples will be stored in the Hematologic Procurement Facility and batch-shipped to Dr. Schwaab's Laboratory for analysis.

Samples will be analyzed in Dr. Schwaab's laboratory:

Buffalo Life Sciences Complex (BLSC)
Center for Genetics and Pharmacology (CGP)
Laboratory location: L5-141
Lab phone: 716-845-7180
Lab contacts:
Mohammad Habiby Kermany, MD PhD-Lab Manager
Jason Muhitch, PhD, Assistant Professor, office number: 716-845-4930
Dr. Muhitch's cell phone number: 716-983-7134

Dr. Schwaab's office number: 716-845-5770
Dr. Schwaab's cell phone number: 716-544-0416

Note: All investigator or analyzing research laboratories housing research samples need to maintain current **Temperature Logs** and study-specific **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

Refer to **Section 13** for correlative data analysis.

9.2 Leukapheresis

Leukapheresis for AGS-003 manufacturing will be performed at the American Red Cross according to the Argos Leukapheresis Manual and sent directly to Argos on the day of collection.

9.3 Nephrectomy

Fresh tissue will be obtained from the surgical specimen to evaluate the effect of AGS-003 treatment. Samples to be taken are primarily tumor tissue, but may also include normal kidney, adrenal gland, lymph nodes, and /or surrounding tissues as included in the resection. The minimum amount needed is 1g and optimum is 10 gramsbut additional amounts will be accepted if not required for other institutional research.

The fresh tissue will be sent to Dr. Schwaab's laboratory for processing:

Buffalo Life Sciences Complex (BLSC)
Center for Genetics and Pharmacology (CGP)
Laboratory location: L5-141
Lab phone: 716-845-7180
Lab contacts:
Mohammad Habiby Kermany, MD PhD-Lab Manager
Jason Muhitch, PhD, Assistant Professor, office number: 716-845-4930
Dr. Muhitch's cell phone number: 716-983-7134
Dr. Schwaab office number: 716-845-5770
Dr. Schwaab cell phone number: 716-544-0416

For historical comparison, the RCC TMA in existence will be used.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current **Temperature Logs** and study-specific **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

Nephrectomy tissue will also be sent to Argos. Kit for tissue will be provided for each patient by Argos. Sample will be sent to CRS Lab Path Team for processing and shipping. Please see Argos Tumor Worksheet

Tumor Specimen Collection Guidelines for Argos

1 The tumor specimen biopsied must represent viable, non-necrotic tissue that is at least 70% tumor

2 Prepare tumor specimen by completing four (4) biopsy passes for each vial of RNA preservative using 16 gauge or 18 gauge biopsy needles.
NOTE: DO NOT place the specimen in formalin or any other fixative.
NOTE: DO NOT use absorbent material while preparing the tumor specimen.

3 Place the tumor specimen into the RNA preservative vial(s), cap, and gently swirl to separate specimen pieces and adequately immerse specimen pieces in preservative.
NOTE: Only use RNA preservative vials provided with this kit.

4 The tumor specimen should be shipped at room temperature the same day as collection. If the shipment cannot go out the same day as collection, the sealed kit with the tumor specimen in it must be stored in a 2-8° C refrigerator until ready for shipment.

9.4 Pathology

9.4.1 Pre-Treatment Formalin-Fixed Paraffin Embedded (FPPE) Biopsy Samples

The following sections of tissue from the most recent (Screening) renal cell tissue biopsy (neoplastic) that exists in the Paraffin Archive in the Department of Pathology will be collected:

- Ten (10) unstained sections cut at 5 microns on plus glass slides.

The study coordinator or designee will be responsible for entering the order into the EMR using the standard format for all clinical trial pathology requirements.

9.4.2 Post-Treatment Resection Formalin-Fixed Paraffin Embedded (FPPE) Samples

The following sections of tissue from the end of treatment nephrectomy (primarily tumor tissue, but may also include normal kidney, adrenal gland, lymph nodes, and /or surrounding tissues) that exists in the Paraffin Archive in the Department of Pathology will be collected:

- Ten (10) unstained sections cut at 5 microns on plus glass slides.

The study coordinator or designee will be responsible for entering the order into the EMR using the standard format for all clinical trial pathology requirements.

Once the samples have been processed by the Pathology Department at RPCI, they will be sent to Dr. Schwaab's laboratory:

Buffalo Life Science Complex (BLSC)
Center for Genetics and Pharmacology (CGP)
Laboratory location: L5-141
Lab phone: 716.845.7180
Lab Contacts:
Mohammed Habiby Kermany
Jason Muhitch, PhD, Assistant Professor, office number: 716-845-4930
Dr. Muhitch's cell phone number: 716-983-7134
Dr. Schwaab office number: 716.845.5770
Dr. Schwaab cell phone number: 716.544.0416

Slides will be shipped by the investigator to Adaptive Biotechnologies, Seattle Washington, for analysis.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current **Temperature Logs** and study-specific **Sample Tracking and Shipping Logs**. The Principal Investigator/Laboratory Manager **must** ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

10 SAFETY EVALUATION

Safety will be assessed through physical examinations, medical history, clinical laboratory tests, vital sign assessments, and evaluation of adverse events (AEs). Assessments will include grading of the frequency and severity of AEs associated with the treatment, including but not limited to clinical laboratory values, autoimmunity evaluations, and evaluations of injection site reactions (ISRs).

10.1 Medical History

During study Pre-treatment, a complete medical history will be obtained that includes medical and oncologic history, demographics (date of birth, sex, race, ethnicity), history of other disease process (active or resolved), medication history, and concomitant illness. The medical history must be documented in the subject's chart.

10.2 Physical Examination

A physical exam that includes a complete review of major body systems and vital signs will be performed by the investigator or designee during Pre-treatment and at the End of Study/Follow-up visit. Vital sign measurements to be obtained include systolic and diastolic blood pressure, heart rate, weight, height (at Pre-treatment only), body temperature, and respiratory rate.

10.3 Autoimmunity Assessments

Autoimmune laboratory parameters (i.e. RF, TG, dsDNA antibody, ANA) will be followed for shifts from Screening assessment on Injection Week #1 (prior to dosing), Injection Week #3

(prior to dosing), Nephrectomy Visit (either at pre-operative visit or on day of nephrectomy prior to surgery), and at End of Study/ Follow-Up visit. Autoimmunity results from the laboratory will be assessed by the investigator for clinical significance. Abnormal laboratory parameters, even as assessed as clinically non-significant, will require closer examination for other indications of clinically manifested autoimmune disease. Abnormal laboratory parameters will be followed by clinical evaluations and repeated laboratory assessments as indicated by the investigator. Clinically confirmed occurrences of autoimmunity will be reported expeditiously to Argos Therapeutics. Any subject who develops autoimmune disease will be withdrawn from the study (refer to **Section 6.5**).

10.4 Injection Site Reaction and Post-Dose Vital Signs Evaluation

Injection sites will be examined for reactions and vital signs assessed 1 hour following AGS-003 administration, while the subject remains in the clinic. Subjects will also be instructed to conduct self-examinations of the injection sites and to contact study staff if a reaction occurs or worsens after a study visit. Mild and moderate ISRs are expected and should resolve without sequelae. Injection site reactions should not be treated with any form of corticosteroids or antihistamines. If desired, non-steroidal anti-inflammatory drugs (NSAIDS) such as ibuprofen may be used to alleviate symptoms.

10.5 Adverse Events

10.5.1 Definition

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding, if clinically significant), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of ‘unrelated’, ‘unlikely’, ‘probable’, or ‘definite’).

An AE is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan in other study-related documents.

10.5.2 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

10.5.3 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.

However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

10.5.4 Abnormal Laboratory Values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia".

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

10.5.5 Preexisting Medical Conditions (Baseline Conditions)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

10.6 Grading and Relationship to Drug

The descriptions and grading scales found in the CTEP Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. The location of the CTCAE CTEP V.4 is:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

AEs not covered by specific terminology listed should be reported with common medical terminology and documented according to the grading scales provided in the CTCAE Version 4.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the patient's clinical state, other therapeutic interventions or concomitant drugs administered to the patient.

- **Unlikely:** The event is doubtfully related to the investigational agent. The event was most likely related to other factors such as the patient's clinical state, other therapeutic interventions, or concomitant drugs.

- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the patient's clinical state, other therapeutic interventions or concomitant drugs.

- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the patient's clinical state, therapeutic interventions or concomitant drugs.

- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the patient's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

10.7 Reporting Adverse Events

Table 2. Guidelines for Routine Adverse Event Reporting for Pilot Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

All routine AEs occurring between the start of intervention until 30 days after the last intervention or until the event has resolved, the study patient is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or reversible, will be reported. New information will be reported when it is received.

10.8 Serious Adverse Events

10.8.1 Definition

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death
- A life-threatening adverse event (experience). Any AE that places a patient or patient, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does NOT include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours). Admission for scheduled nephrectomy will not be considered an SAE.
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly or birth defect
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above

10.8.2 Reporting Serious Adverse Events

All new SAEs occurring from the date the patient signs the study consent until 30 days after the last intervention or a new treatment is started, whichever comes first, will be reported. The RPCI SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information.

SAE's occurring after the 30 day follow-up period that the investigator determines to be possibly, probably or definitely related to the study intervention, should be reported.

SAE's identified as an Unanticipated Problem by the Investigator must be reported. Please refer to **Section 10.9.2** for details on reporting Unanticipated Problems.

10.8.3 Investigator Reporting: Notifying the AGS-003 manufacturer

SAEs that meet requirements for expedited reporting (probably or definitely related to AGS-003) and are inconsistent with the safety profile in the current AGS-003 IB must be rapidly reported to Argos.

Argos Therapeutics, Inc.
4233 Technology Drive
Durham, NC 27704-2173
Ph. 919-287-6300
Fax 919-287-6301

10.8.4 Follow-Up for Serious Adverse Events

All related SAEs should be followed to their resolution, the study patient is lost to follow-up, the start of a new treatment, or until the study investigator assesses the event(s) as stable or irreversible. New information will be reported when it is received.

10.9 Unanticipated Problems

10.9.1 Definition

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
  - The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of patient privacy or confidentiality of data.
  - The characteristics of the patient population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places patients or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed serious per Section 10.8.

10.9.2 Reporting Unanticipated Problems

Unanticipated problem reporting will begin at the time of participant consent. The Unanticipated Problem Form will be submitted to CRS Compliance Office within 1 business day of becoming aware of the Unanticipated Problem. After review, CRS Compliance will submit the UP to the IRB.

When becoming aware of new information about the Unanticipated Problem, submit this updated information to CRS Compliance with an updated Unanticipated Problem Form. The site Investigator or designated research personnel will report all unanticipated problems, whether related or unrelated to the investigational agent(s) to the **IRB in accordance with their local institutional guidelines**.

10.10 FDA Reporting

When RPCI is the IND holder the following describes the FDA reporting requirements by timeline for AEs and new safety findings that meet the criteria outlined below:

Within 7 Calendar Days

Any adverse event that meets ALL the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Fatal or life-threatening.

Within 15 Calendar Days

Any adverse event that meets ALL the following criteria:

- Related or possibly related to the use of the study drug;
- Unexpected; and
- Serious but not fatal or life-threatening.

Or, meets ANY of the following criteria:

- A previous adverse event that is not initially deemed reportable but is later found to fit the criteria for reporting (report within 15 days from when event was deemed reportable).
- Any findings from other studies, including epidemiological studies, pooled analysis of multiple studies, or other clinical studies conducted with the study drug that suggest a significant risk in humans exposed to the drug.
- Any findings from animal or in vitro testing that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity or reports of significant organ toxicity at or near the expected human exposure.
- Any clinically important increase in the rate of occurrence of a serious, related or possibly related adverse event over that listed in the protocol or investigator brochure.

Sponsors are also required to identify in IND safety reports, all previous reports concerning similar adverse events and to analyze the significance of the current event in the light of the previous reports.

Reporting Process

The principal investigator or designee will complete and submit a FDA Form 3500A MedWatch for any event that meets the above criteria. Forms will be submitted to the CRS Compliance Office via email to CRSCompliance@RoswellPark.org.

10.11 Gene Therapy Study Reporting of Serious Adverse Events

Studies that involve Gene Therapy will report SAEs as described above and will be reported to the Office of Biotechnology Activities (OBA) / NIH.

Any SAE that is fatal or life-threatening, that is unexpected, and associated with the use of the gene transfer product must be reported to the FDA and NIH OBA as soon as possible, but not later than 7 calendar days after the Sponsor's initial receipt of the information.

SAEs that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, must be reported to the FDA and NIH OBA as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information.

If, after further evaluation, an adverse event initially considered not to be associated with the use of the gene transfer product is subsequently determined to be associated, then the event must be reported to the NIH OBA within 15 days of the determination.

Any follow-up information relevant to a SAE must be reported within 15 calendar days of the Sponsor's receipt of the information. If a SAE occurs after the end of a clinical trial and is determined to be associated with the use of the gene transfer product, that event shall be reported to the NIH OBA within 15 calendar days of the determination.

The Principal Investigator or delegated research staff will submit a report to the CRS Compliance Office via email: CRS Compliance for all gene therapy SAEs. The Compliance office will forward the information to ORSP.

Completed reports may be sent via U.S. mail, courier service, e-mail, or facsimile to:

NIH Office of Biotechnology Activities
6705 Rockledge Drive, Suite 750
Bethesda, Maryland 20892-7985
(For all non-USPS deliveries use Zip Code 20817)
Telephone: 301-496-9838
Fax: 301-496-9839
E-mail address for Reporting Adverse Events: GeMCRIS@od.nih.gov
General E-mail: oba@od.nih.gov
Website: http://oba.od.nih.gov/oba/index.html

Additional information for reporting adverse events, including a report template, can be found at: http://oba.od.nih.gov/rdna/adverse_event_oba.html.

11 DATA AND SAFETY MONITORING

The RPCI Data and Safety Monitoring Board will assess the progress of the study, the safety data, and critical efficacy endpoints. The DSMB will review the study annually and will make recommendations that include but not limited to; (a) continuation of the study, (b) modifications to the design (c) or termination of the study.

12 STATISTICAL METHODOLOGY

The primary objective of this study is to assess immune-modulatory systemic and intratumoral effects of AGS-003 as neoadjuvant treatment in patients with localized renal cell carcinoma.

The time component will be modeled as a three-level classification factor. The full model for the effects of time will be fit using Linear Mixed Model methods. The model will include a random patient effect and 5 fixed effects for time and the interactions. The presence of any time effect will be assessed with full-reduced model Type 3 test. If the omnibus test is statistically

significant at the $p < 0.05$ level, then three pairwise time-point comparisons will be conducted. Expression measurements may be transformed to satisfy modeling assumptions. This methodology accommodates patients with incomplete follow-up information.

Patient, disease and treatment characteristics will be summarized using common descriptive statistics and graphical aids. P values less than 0.05 will be deemed statistically significant. No adjustments will be made to control the overall Type I error rate.

12.1 Sample Size Determination

A maximum of 10 patients will be enrolled in this study. In previous experiments, immune marker expression measurements varied widely, with means between 8.0 and 1,200; the coefficient of variation has been between 0.5 and 1.0 depending on the marker, method of measurement and disease setting. For power calculations, the possible effect of immunotherapy delivery model of expression has been ignored, and the (original scale) expressions are assumed to have mean (standard deviation) of 100 (75), and within-patient correlation = 0.50. If the measurements are log-normally distributed, the log-transformed outcome would have mean (standard deviation) of about 4.38 (0.68).

Comparing measurements at two time points, the within-patient effect of immunotherapy on an immune marker can be assessed using a permutation paired t-test. A paired t-test with a two-sided significance threshold of 0.05 has 80% power to detect a mean of at least 5.08 in the post-measurements, if the pre-measurements are as described above: a 20% increase in the mean on the original scale.

12.2 Efficacy Analysis

The efficacy analysis discussed above will be conducted using all patients with at least two time point measurements.

12.3 Safety Analysis

Adverse event rates will be summarized in all patients who received AGS-003. These rates will be described as the proportion of patients with the event, by grade, and supported with exact 95% confidence intervals.

12.4 Stopping Rule:

If during the trial 4 or more patients experience the undesirable event defined as unexpected bleeding or more difficult than usual perinephric dissection, accrual will be halted and the treatment will be deemed unsafe. Otherwise the accrual will continue for a total of 10 patients. The probability of excising the stopping rule within this study is a function of the true unknown probability of the event corresponding to the study population. The table below contains the probability of observing at least 4 undesirable events in the 10 patients for a variety of values of the true probability within the population.

True probability of undesirable event	0.2	0.3	0.4	0.5
Probability of exercising the stopping rule	0.12	0.35	0.62	0.83

The calculations above are based on the assumption that our study sample may be treated as a random sample from the population of interest.

12.5 Interim Analysis and Criteria for Early Termination of the Study

No explicit interim analyses are planned for this study, given the low stage and slow growth rate of this type of cancer.

13 CORRELATIVE DATA ANALYSIS

Immune parameters will be assessed to demonstrate the effect of AGS-003 on the immune system. Immune effector cell populations will be assessed in a very detailed fashion and will focus on those populations that were found to be of interest in previous studies with AGS-003. Therefore, this study will analyze $CD8^{+}CD28^{+}$ T cells (REHA), In addition, this study will analyze T-regulatory cells (CD4+CD25+FoxP3+) and myeloid derived suppressor cells (MDSC) using multicolor flow cytometry. These cell populations will be assessed in peripheral blood specimens as well as in tumor lysates. The serum cytokine microenvironment will be assessed using multiplex Th1/Th2 cytokine ELISA (this will include IL-12 and IFN-γ). If perinephric lymph nodes are removed as standard of care during the kidney surgery, these will be procured and analyzed in a similar fashion. Autologous T cells will be incubated with irradiated autologous tumor cells and T cell proliferation will be measured in IFN-γ ELISPOT assays.

After appropriate tumor tissue has been procured to assure pathologic diagnosis, tumors will be processed into tumor lysates. These lysates will be analyzed using flow cytometry as described above. The tumor infiltrating immune cell populations and tumor cytokine microenvironment will be assessed using standard double-staining immunohistochemistry. These results will be compared to a historical control tissue microarray (TMA) that contains 264 RCC specimens.

14 ETHICAL AND REGULATORY STANDARDS

14.1 Ethical Principles

This study will not be initiated until the protocol and informed consent document(s) have been reviewed and approved by a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Each patient (or legal guardian) shall read, understand, and sign an instrument of informed consent prior to performance of any study-specific procedure. It is the responsibility of the investigator to ensure that the patient is made aware of the investigational nature of the treatment and that informed consent is given.

The Investigator is responsible for the retention of the patient log and patient records; although personal information may be reviewed by authorized persons, that information will be treated as strictly confidential and will not be made publicly available. The investigator is also responsible for obtaining patient authorization to access medical records and other applicable study specific information according to Health Insurance Portability and Accountability Act regulations (where applicable).

This study will be conducted in compliance with all applicable laws and regulations of the state and/or country and institution where the patient is treated. The clinical trial should be conducted in accordance with the ethical principles embodied in the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, consistent with good clinical practice and the applicable regulatory requirements and according to the guidelines in this protocol, including attached appendices

14.2 Informed Consent

The Investigator is responsible for obtaining written consent from each patient or the patient's legally authorized representative in accordance with GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the patient according to applicable GCP guidelines, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional information that is applicable to the study must also be included. Additional national or institutionally mandated requirements for informed consent must also be adhered to. The patient should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator shall provide a copy of the signed consent form to the patient and the signed original shall be maintained in the Investigator File. A copy of the signed consent form must be filed in the patient file. At any stage, the patient may withdraw from the study and such a decision will not affect any further treatment options.

15 STUDY RESPONSIBILITIES

15.1 Data Collection

Data entry into the database is to be completed in a timely fashion within 30 days after the patient’s clinic visit. If an AE is considered serious it is captured on both the Adverse Event page and the Serious Adverse Event Source Form, which is handled in an expedited fashion.

Data management activities will be performed using eClinical. eClinical is a suite of software tools that enables the collection, cleaning and viewing of clinical trial data. CRS data management will design the study-specific database and facilitate its development by the eClinical Information Technology team. Once the database design is approved by the Investigator, Statistician, and Clinical Research Coordinator, the database will be put into production and data entry can begin. Data can be entered and changed only by those with the

rights to do so into electronic case report forms (eCRFs). eClinical is compliant with all technical aspects of relevant GCP guidelines.

- The system can generate accurate copies of stored data and audit trail information in human readable form.
- System access is limited to authorized individuals through the controlled assignment of unique ID and password combinations.
- The system is designed to periodically force users to change their passwords and verifies that user ID and password combinations remain unique.
- The system automatically generates a permanent time-stamped audit trail of all user interactions.

When data entry is complete, data management will review the data and will query any missing, incomplete, or invalid data points for resolution by the Clinical Research Coordinator and Investigator. Once all queries have been resolved, the data can be released to the statistician for analysis.

15.2 Maintenance of Study Documents

Essential documents should be retained per RPCI's policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with RPCI.

16 ADMINISTRATIVE RULES

16.1 Revisions to the Protocol

RPCI may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the IRB/ERC for written approval before implementation.

16.2 Termination of the Study

It is agreed that, for reasonable cause, either the RPCI Investigator or the AGS-003 manufacturer (Argos), may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, RPCI may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of patients enrolled in the study.

16.3 Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

17 APPENDICES

Appendix A ECOG Performance Status Scores

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

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