Official Study Title:

A Phase II Trial of Neoadjuvant AGEN1884 plus AGEN2034 in Combination with Cisplatin-Gemcitabine for Muscle-Invasive Bladder Cancer Prior to Radical Cystectomy

(CTMS# 19-0193)

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Protocol Version 10 dated 2/23/21

Summary of Changes

Cover Page
Updated cover with current version change
Reason: Administrative change
Global
Minor edits throughout
Reason: correct spelling, alignment, grammar
Study Summary
Added: **If the total number of pCR is ≤13, the combination will be deemed as failing to meet the primary objective.**
Reason: To clarify, per statistical review.
Study Sample Size/ 5. STATISTICAL CONSIDERATIONS
We will submit 17 patients to treatment and fail to reject the null hypothesis and terminate the study unless at least ~~4~~ **5**/17 pCRs are observed. If at least ~~4~~ 5 pCRs are observed, we will submit an additional 19 patients to treatment. If at least ~~13~~ **14**/36 pCRs are observed we will reject the null hypothesis in favor of the sought improvement in success rate. This modified Simon two-stage design yields a type I error rate of 0.044 and power of 0.8 when the true response rate is 0.45.
Reason: Correction, increase by 1 in each section, to align with the original statistical design
Study Eligibility

Absolute neutrophil count	~~>~~ ≥ 2,000/mcL
Hemoglobin	~~>~~ ≥ 9.0 mg/mL
Platelets	~~>~~ ≥ 100,000/mcL
Total bilirubin	within normal limits or known to be elevated due to a benign conjugation defect such as

		Gilbert's syndrome, as evidenced by normal conjugated bilirubin level
	AST/ALT	< 3X institutional normal limits
	Creatinine clearance (CrCl)	≥ 50 mL/min/1.73m2, as measured with 24 hr urine collection or estimated by CKD-EPI, whichever is greater

Reason: Correcting values to be consistent throughout protocol

4.1.1 Safety Run-In

The safety run-in of the study will first enroll three patients ~~at the chosen dose and schedule of the combination~~ **who will begin treatment with cisplatin and gemcitabine plus AGEN2034 and AGEN1884 as outlined in the treatment plan**. These first 3 patients will be ~~followed to completion of day 1 cycle 2 of neoadjuvant therapy in the final patient in this cohort prior to enrollment and~~ assessed for DLTs **and there will be a pause in enrollment until all three complete the DLT period**. DLT is defined from events experienced from day 1, cycle 1 through the ~~day before~~ day 1, cycle 2. If there are no DLTs in the first 3 patients, we will proceed to further accrual to stage I of phase II. If there is 1 DLT in the initial 3 patients, we will enroll 3 additional patients to the safety run-in. If ~~a second~~ **≥ 2** DLT**s** ~~is~~ **are** experienced **in the initial 3 patients**, the study will be terminated. If there ~~is~~ **are** only ~~1~~ **2** DLT**s** in the 6 patients treated, further accrual can proceed. **If there are >2 DLTs in the 6 patients treated, the study will be halted.** All patients who receive at least two cycles of neoadjuvant therapy will be eligible for the primary efficacy endpoint of the study. Those who do not receive cystectomy or who receive <2 cycles of neoadjuvant therapy will not be evaluable for the primary efficacy endpoint and must be replaced.

Reason: Provide clarification

4.1.2 Phase II, Stage 1

The study will proceed to the second stage provided the early stopping rules have not been met and at least ~~4~~ **5** adequately treated patients achieve a pCR. Evaluable patients are defined as those receiving at least 2 cycles of neoadjuvant immunotherapy plus chemotherapy as scheduled.

Reason: Correction

5.1 Study Design/Endpoints/ 8. References

Added:

The number screened, the number of screen failures by reason, the number enrolled, and the number lost to follow-up reason will be tabulated. The Simon two-stage design results will be descriptively summarized. Secondary end points will be descriptively summarized. Progression free survival will be described with a graphic of the Kaplan-Meier curve, the median time to progression, and a 95% confidence interval for the median time to progression. Tabular summaries, graphics, and the median time to progression will be provided with R [30].

30. R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
Reason: Describing statistical calculations.
4.3 Treatment Administration
Added: **Cisplatin and gemcitabine will be prepared and administered as per standard of care. AGEN2034 and AGEN1884 will be prepared and administered as per the Agenus pharmacy manual for each agent, respectively.**
Reason: Addition of pharmacy manuals to study.

A Phase II Trial of Neoadjuvant AGEN1884 plus AGEN2034 in Combination with Cisplatin-Gemcitabine for Muscle-Invasive Bladder Cancer Prior to Radical Cystectomy
(CTMS# 19-0193)

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CTMS# 19-0193

IND# 142737

PROTOCOL V10

DATE: 2/23/21

INVESTIGATOR'S AGREEMENT

I have read and understand the contents of this clinical protocol for Protocol: **A Phase II Trial of Neoadjuvant AGEN1884 plus AGEN2034 in Combination with Cisplatin-Gemcitabine for Muscle-Invasive Bladder Cancer Prior to Radical Cystectomy** and will adhere to the study requirements as presented, including all statements regarding confidentially. In addition, I will conduct the study in accordance with current international conference on harmonization (ICH) guidance, Good Clinical Practice (GCP) guidance, the Declaration of Helsinki, US Food and Drug Administration (FDA) regulations and local IRB and legal requirements.

Name of Investigator: Chethan Ramamurthy, MD

Institution: Mays Cancer Center, UT Health San Antonio

_____ _____

Investigator Signature Date

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1. SYNOPSIS

Trial Title	**A Phase II Trial of Neoadjuvant AGEN1884 plus AGEN2034 in Combination with Cisplatin-Gemcitabine for Muscle-Invasive Bladder Cancer Prior to Radical Cystectomy**
Trial Number	CTMS# 19-0193
Sponsor	Mays Cancer Center, UT Health San Antonio
Phase	Phase II
Names of Investigational Products	AGEN1884 AGEN2034
Number of Study Sites	1 (United States)
Planned Number of Subjects	Phase II: 36 subjects
Study Population	Subjects ≥18 years of age who have muscle invasive urothelial cancer of the bladder eligible for neoadjuvant cisplatin-based chemotherapy prior to radical cystectomy
Study Summary	This is a Phase II trial to establish safe and tolerable AGEN1884 plus AGEN2034 doses in combination with neoadjuvant cisplatin and gemcitabine to enable stage 1 of a Simon 2-stage trial to evaluate clinical benefits of the addition of neoadjuvant AGEN1884 plus AGEN2034 concurrent with cisplatin and gemcitabine neoadjuvant chemotherapy in subjects who have muscle-invasive, non-metastatic (cT2-4N0-1M0) urothelial bladder cancers and are eligible for cisplatin-based neoadjuvant chemotherapy prior to radical cystectomy. The study will begin with a 3+3 design safety run-in to establish the safety of this combination, with a minimum of 6 and a maximum of 9 patients. The Stage 1 portion of the Simon 2-stage design phase II trial will enroll 17 subjects based on assumptions and calculations set forth. The Simon 2-stage design halts the trial for futility for ≤ 4 tumor downstage responses in the first 17 patients. Otherwise, an additional 19 patients will receive AGEN1884 plus AGEN2034 concurrent with cisplatin and gemcitabine chemotherapy in stage 2. If the total number of pCR is ≤13, the combination will be deemed as failing to meet the primary objective. Each experimental treatment cycle is 21 days and matched to the 21-day chemotherapy cycles. AGEN2034 is given on day 1 of each cycle, for a total of 4 chemoimmunotherapy cycles, whereas AGEN1884 is given on day 1 of cycles 1 and 3 only, for a total of 2 cycles, followed in no longer than 10 weeks by radical cystectomy. The primary endpoint is pathologic tumor downstaging of ≥T2 to T0 and is compared to historical controls. Assuming a historical 25% rate of downstaging, enrollment of 36 total patients in the full phase II trial has 80% power to detect a 45% pathologic complete response rate in the phase II portion of the trial.

Trial Objectives	**Primary Objectives** • To estimate the pathologic tumor downstaging of ≥T2 to pT0 rate using neoadjuvant AGEN1884 plus AGEN2034 in combination with cisplatin-gemcitabine chemotherapy in the treatment of muscle-invasive bladder cancer in patients eligible for cisplatin-based chemotherapy. **Secondary Objectives** • To evaluate the safety and tolerability of using AGEN1884 plus AGEN2034 plus cisplatin and gemcitabine chemotherapy in the neoadjuvant treatment of muscle-invasive bladder cancer prior to radical cystectomy • To estimate pathologic downstaging of ≥T2 to <T2 rate • To investigate biological outcomes that could predict treatment efficacy **Exploratory Objective** • To correlate clinical outcomes with immune and biologic endpoints and identify patient and tumor characteristics that can predict treatment responses
Background	**1.1 Muscle Invasive Bladder Cancer (MIBC)** Bladder cancer (BC) is the fourth most common malignancy in US men, with 79,030 new annual cases causing an estimated 16,780 deaths in the US in 2017. The majority of cases are superficial tumors, managed by local therapies. However, once muscle invasion is identified outcomes are much poorer, with approximately one-half of patients dying of their disease.[1] For patients with MIBC, surgery alone achieves a poor outcome, with a 5-year survival rate of 40-50%. Neoadjuvant cisplatin-based chemotherapy regimens have demonstrated a modest but absolute benefit in overall survival of 5-8% compared with surgery alone. Thus, while the standard of care in the treatment of MIBC is neoadjuvant cisplatin-based chemotherapy followed by radical cystectomy, there is a need for improvement. **1.2 Neoadjuvant Chemotherapy for MIBC** A pivotal trial randomized patients to three 28-day cycles of neoadjuvant MVAC (methotrexate, vinblastine, Adriamycin, cisplatin) plus cystectomy versus cystectomy alone, and found a 14% (p=0.06) improvement in overall survival in the combination therapy group, establishing neoadjuvant cisplatin-based chemotherapy as the standard of care.[2] The pathologic complete response (pCR) rate was 38% in the neoadjuvant MVAC arm. However, the classic MVAC regimen carried a high rate of grade 3/4 toxicity. Subsequent studies have refined the platinum containing regimen. After a study demonstrating improved toxicity with a dose-dense schedule of MVAC (ddMVAC) in metastatic urothelial cancer, two single arm phase II studies have demonstrated comparable pCR rates with neoadjuvant ddMVAC and classic MVAC. One study utilized four cycles of ddMVAC and found a pathologic response rate of 49%, with a pCR rate of 26%.[3] Another study used three cycles of ddMVAC, which yielded a pathologic response rate of 53% and a pCR rate of 38%.[4] These studies have led many experts and consensus guidelines such as the National Comprehensive Cancer Network (NCCN) to recommend a dose dense schedule of MVAC over classic MVAC.[5] However, the multidrug MVAC regimen is not suitable for all patients, particularly the elderly bladder cancer patient with potential for cardiovascular comorbidities. Gemcitabine was found to be an active agent in advanced or metastatic urothelial cancer,[6] and a randomized phase III trial in patients with metastatic urothelial cancer comparing gemcitabine-cisplatin (GC) to classic MVAC found improved toxicity and similar efficacy with GC.[7,8] This use of GC has been extrapolated to the neoadjuvant setting, and large population based studies indicate that of those receiving neoadjuvant chemotherapy for MIBC, the most commonly used regimen is

GC.[9] The NCCN also includes GC among the preferred perioperative chemotherapy regimens for MIBC.[5]

There are two primary dosing schedules for GC, a 21-day schedule and a 28-day schedule. The 21-day schedule was found to have similar dose intensity and better compliance profile than the 28-day schedule in a randomized phase II trial, so it is commonly preferred.[10]

1.3 Checkpoint Inhibition in BC

BC tumors typically carry among the highest cancer mutational loads known,[11] which correlate with the best responses to immunotherapies in other cancers.[12] Thus, the rationale for BC immunotherapy is strong, further evidenced by recent FDA approvals of two types of BC immunotherapies, antibodies blocking PD-L1 (αPD-L1) or PD-1 (αPD-1) immune checkpoint molecules.

PD-L1 is an immune checkpoint molecule that negatively regulates T cell functions through its known receptors PD-1 and CD80,[13] and is immunopathogenic in many cancers.[14] αPD-L1 has demonstrated remarkable clinical efficacy against a variety of cancers, including recent FDA-approvals of αPD-L1 for BC. PD-1 is also an immune checkpoint molecule and αPD-1 is also FDA-approved to treat BC. Despite major efficacy in a selected few patients, these immune checkpoint inhibitor antibodies are not typically curative or universally effective, with an average ~30% response rate. CTLA-4 is the third immune checkpoint molecule for which an immune checkpoint inhibitor antibody is FDA-approved (melanoma, renal cell carcinoma). While αPD-1 has significant activity as monotherapy in metastatic BC, the combination of αPD-1 and αCTLA-4 at selected doses led to even higher response rates and improvements in survival for patients with pretreated metastatic urothelial carcinoma.[15]

Combinations of two immune checkpoint inhibitors have demonstrated superior response rates to either single agent. For example, the combination of αPD-1 plus αCTLA-4 is FDA-approved for selected melanomas, as the combination provides superior response rates to either single agent.[12] The combination is also approved for the treatment of renal cell carcinoma.[16] Likewise, αPD-1 plus αCTLA-4 provides superior response rates to either single agent to treat certain non-small cell and small cell lung cancers.[17,18]

1.4 Rationale for Combination Checkpoint Inhibition plus Chemotherapy

The most successful combination of chemotherapy with checkpoint inhibitors thus far has been in lung cancer. The KEYNOTE-021 study was a randomized phase II trial of chemotherapy-naïve advanced non-squamous, non-small cell lung cancer patients.[19] Based on phase I data showing the highest ORR with the combination of carboplatin and pemetrexed with pembrolizumab, this trial randomized patients to receive either 4 cycles of carboplatin-pemetrexed with continued pemetrexed maintenance alone or the same regimen combined with pembrolizumab 200 mg every 3 weeks for 24 months. The pembrolizumab plus chemotherapy group (n = 60) had an improved ORR of 55% (95% CI, 42-68%) compared with the chemotherapy alone group (n = 63), which had an ORR of 29% (95% CI, 18-41%). Median PFS in the combination group was 13.0 months (95% CI, 8.3-not reached) compared with 8.9 months (95% CI, 4.4-10.3) in the chemotherapy alone group. The rate of grade 3 or higher AEs was slightly higher with the combination (39%) than with chemotherapy alone (26%) but did not lead to an increased discontinuation rate (10% versus 13%, respectively). Interestingly, responses were seen regardless of tumor PD-L1 expression, with 57% ORR (95% CI, 34-79) in the PD-L1 <1% group and 54% (95% CI, 37-70) in the PD-L1 $\geq$1% group.

GC chemotherapy is an established standard of care in the treatment of MIBC, as detailed above. Furthermore, both agents have been shown to enhance antitumoral immune activity. Cisplatin can upregulate MHC class I expression, promote recruitment and proliferation of effector T cells, downregulate immunosuppressive elements of the tumor microenvironment, and upregulate activity of cytotoxic effectors.[20] Similarly, gemcitabine too has been shown to

	increase antigen presentation and MHC class I expression as well as reduce population of myeloid-derived suppressor cells *in vitro*.[21] The regimen is also most effectively administered on a 21-day schedule, similar to the investigational agents. Thus, this is an ideal chemotherapy backbone for this study of combination chemoimmunotherapy. **1.5 Rationale for pT0 as the Clinical Endpoint** Pathologic downstaging to pT0 in BC at cystectomy is strongly associated with long-term disease-free survival. For patients with pT0 at time of cystectomy (pathologic complete response or pCR), 5-year overall survival was 85%, compared to only 45% for patients with residual disease at time of cystectomy.[22] This endpoint has been used to justify dose-dense MVAC as a standard of care based on results of a phase II clinical trial, and is currently used as the primary endpoint of several ongoing neoadjuvant cystectomy trials.[4] The reported pCR rates to cisplatin-based neoadjuvant chemotherapy vary widely, ranging from 15-41%.[4,23-26] A large, recent cross-sectional analysis of 332 patients treated at an academic institution who received neoadjuvant chemotherapy prior to cystectomy reported a pCR rate of 24.5% in the 204 patients receiving neoadjuvant GC.[26]
Study Sample Size	The study will begin with a safety run-in. Three patients will be treated with the combination and observed for a DLT during the first cycle of treatment. After no DLT is observed in the first 3 patients, an additional 3 patients will be treated and observed for DLTs. If the specified safety criteria are met, the study will proceed to the full Simon two-stage design. The overall study employs a modified Simon two-stage design with a minmax design, enrolling 17 patients in stage I and subsequently 19 more patients in stage II if early termination criteria are not met. There are early termination criteria for futility. To be evaluable for the primary endpoint in phase II, patients must have received at least two cycles of neoadjuvant therapy, otherwise they must be replaced. Using a historical pCR rate of 0.25 as the null hypothesis, we will seek to demonstrate a rate of 0.45. The reported pCR rates to cisplatin-based neoadjuvant chemotherapy vary widely, ranging from 15-41%[4,23-26]. We have chosen a rate of 25% based on the pCR rate for GC from a large, recent cross-sectional analysis of 332 patients who received neoadjuvant chemotherapy prior to cystectomy.[26] We will submit 17 patients to treatment and fail to reject the null hypothesis and terminate the study unless at least 5/17 pCRs are observed. If at least 5 pCRs are observed, we will submit an additional 19 patients to treatment. If at least 14/36 pCRs are observed we will reject the null hypothesis in favor of the sought improvement in success rate. This modified Simon two-stage design yields a type I error rate of 0.044 and power of 0.8 when the true response rate is 0.45.
Study Eligibility	**Inclusion Criteria** 1. Diagnosis of muscle-invasive, non-metastatic urothelial carcinoma of the bladder, cT2-4, N0-1, M0 2. Eligible to receive cisplatin-based chemotherapy, with eligibility defined as meeting all of the following criteria: a) Eastern Cooperative Oncology Group performance status of 0-1 b) Creatinine clearance (CrCl) of ≥50 mL/min, as measured by 24-hour urine collection or estimated by the CKD-EPI equation. Patients with CrCl between 50 – 60 mL/min are eligible for the study but will receive split dose cisplatin c) Grade ≤2 hearing loss d) Grade ≤2 peripheral neuropathy e) New York Heart Association Class < III congestive heart failure

3. Eligible to receive gemcitabine as dosed here
4. Patients must have organ and marrow function meeting the criteria below:

Absolute neutrophil count	≥ 2,000/mcL
Hemoglobin	≥ 9.0 mg/mL
Platelets	≥ 100,000/mcL
Total bilirubin	within normal limits or known to be elevated due to a benign conjugation defect such as Gilbert's syndrome, as evidenced by normal conjugated bilirubin level
AST/ALT	< 3X institutional normal limits
Creatinine clearance (CrCl)	≥ 50 mL/min/1.73m2, as measured with 24 hr urine collection or estimated by CKD-EPI, whichever is greater

5. Signed, written informed consents to allow transfer of tumor tissue and production of peptides and to receive experimental treatment and monitoring if agreeable, or monitoring without experimental treatment otherwise
6. Age ≥18 years
7. Available fresh tissue from surgical excision. If fresh tissue is not available, archival tissue may be used.
8. Female subjects of childbearing potential must have a negative serum pregnancy test at screening (within 72 hours of first dose of study medication). Non-childbearing potential (other than by medical reasons) is defined as 1 of the following:
    a) ≥45 years of age and amenorrheic for >1 year by self-report.
    b) Amenorrheic for >2 years without a hysterectomy and oophorectomy, and follicle-stimulating hormone value in the postmenopausal range upon pretrial (screening) evaluation.
    c) Status post-hysterectomy, -oophorectomy, or –tubal ligation.
9. If of childbearing potential, female subjects must be willing to use adequate birth control during the study, starting with the screening visit through 120 days after the last dose of study therapy.
10. Male subjects with a female partner(s) of childbearing potential must agree to use a condom throughout the trial, starting with the screening visit through 120 days after the last dose of study therapy. Males with pregnant partners must agree to use a condom; no additional method of contraception is required for the pregnant partner.

Note: Abstinence is acceptable for both female and male subjects if this is the subject's established and preferred contraception method.

Exclusion Criteria

1. Subjects must not have previously received a checkpoint inhibitor ie, anti-PD-1, anti-PD L1, or anti CTLA-4 antibody.
2. Subjects must not have previously received anticancer medications or investigational drugs for the disease under study within the following windows:

	a. ≤ 28 days for prior monoclonal antibody used for anticancer therapy, with the exception of denosumab b. ≤ 7 days for immunosuppressive treatment for any reason, with the following exceptions: i. Physiologic steroid replacement for adrenal insufficiency (e.g., <10 mg prednisone per day) is permitted. ii. Use of inhaled or topical corticosteroid for radiographic procedures is permitted. c. Systemic corticosteroids ≤ 7 days are not allowed except as defined above. d. ≤ 28 days before first dose of study drug for all other investigational study drugs or devices 3. Has persisting toxicity related to prior therapy of National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (NCI-CTCAE) Grade >1 severity. Note: Sensory neuropathy or alopecia of Grade ≤2 is acceptable. 4. Has known severe hypersensitivity reactions to fully human monoclonal antibodies (NCI-CTCAE Version 5.0 Grade ≥3), any history of anaphylaxis, or uncontrolled asthma. 5. Active or history of any autoimmune disease (subjects with diabetes type 1, vitiligo, psoriasis, hypo- or hyperthyroid disease not requiring immunosuppressive treatment are eligible). Patients with a history of inflammatory bowel disease (including Crohn's disease and ulcerative colitis) and autoimmune disorders such as rheumatoid arthritis, systemic progressive sclerosis [scleroderma], Systemic Lupus Erythematosus or autoimmune vasculitis [e.g., Wegener's Granulomatosis] are excluded from this study. 6. Any condition requiring systemic treatment with corticosteroids (>10mg daily prednisone equivalents) or other immunosuppressive medications within 14 days prior to first dose of study drug. Inhaled steroids and adrenal replacement steroids doses >10mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. 7. Uncontrolled intercurrent illness, including but not limited to uncontrolled infection, interstitial lung disease or active, non-infectious pneumonitis, symptomatic congestive heart failure, unstable angina pectoris, uncontrolled cardiac arrhythmia, or social situations that would limit compliance with study requirements in the opinion of the treating investigator or medical monitor. 8. History of intolerance or allergic reactions attributed to compounds of similar chemical or biologic composition to AGEN1884 or AGEN2034. 9. Women who are pregnant or breastfeeding. 10. Receipt of a live vaccine within 30 days prior to the first dose of study drug. 11. Inability to adhere to the protocol.
Dosage and Administration	AGEN1884 1mg/kg q6 weeks plus AGEN2034 300mg q3 weeks with cisplatin 70 mg/m2 day 1 and gemcitabine 1000 mg/m2 days 1 and 8 of a 21 day cycle to a maximum of 4 cycles. AGEN1884 plus AGEN2034 are given on day 1 of 21-day cycles 1 and 3 and AGEN2034 is given on day 1 of cycle 2, after cisplatin and gemcitabine. AGEN1884 is given before AGEN2034 when both are given on the same day.
Planned Trial Duration	Subject accrual: Estimated 2 years Study duration: ≥60-64 weeks/subject • Screening: 2-4 weeks

	• Experimental treatment period: 12 weeks • Follow-up period:2 years
Schedule of Visits and Assessments	**Screening** This phase will take approximately 2 weeks/patient **Treatment and Evaluation Phase** The treatment and evaluation phase for patients undergoing experimental treatments consists of cycles with cisplatin and gemcitabine chemotherapy plus concurrent AGEN1884 and/or AGEN2034, plus additional periodic immune, biologic and clinical monitoring. *Safety*: Safety will be assessed at every visit by frequency, severity, and duration of treatment-emergent adverse events per National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0, physical examinations, vital signs, weight, clinical laboratory tests, and ECOG performance status. Collection of adverse events will begin at time of first study treatment. *Clinical*: Disease evaluations will be conducted prior to the initial dose of study treatment, and every 3 weeks during experimental treatments. Prior to Cycle 3 Day 1, restaging imaging evaluation will be performed. At the end of experimental treatment, patients will proceed to radical cystectomy and be evaluable for the primary endpoint of pathologic complete response (pT0). *Immunogenicity*: To determine induction of subject's immunoreactivity against bladder cancer **Follow-up Phase** *End-of-Treatment (or Discontinuation) Visit (14 days ± 5 days after last treatment)* Subjects who discontinue experimental treatment prematurely for an adverse event will undergo a full safety evaluation at the time of discontinuation and will be followed for survival for 1 year from first study treatment. *Final Safety Visit* A final safety visit may be performed 30 (± 7) days after the last experimental treatment administration at the discretion of the investigator. Subjects with an ongoing adverse drug reaction at this visit must be followed until the event resolves, becomes stable, or is considered not to be clinically significant by the investigator. *Survival Follow-up* Subjects will be followed (by phone or office visit) every 12 weeks from their cystectomy for 2 years to assess survival.
Endpoints	**Primary Endpoints** • Pathologic tumor downstaging of ≥T2 to pT0 **Secondary Endpoints** • CTCAE v. 5.0 • Pathologic downstaging to <T2 • Completion of surgery • Progression-free survival at 1 year **Exploratory Endpoints** • Correlative immune outcomes

2. OBJECTIVES

2.1 Primary Objectives

- To estimate the pathologic tumor downstaging of ≥T2 to pT0 rate using neoadjuvant AGEN1884 plus AGEN2034 in combination with cisplatin-gemcitabine chemotherapy in the treatment of muscle-invasive bladder cancer in patients eligible for cisplatin-based chemotherapy

2.2 Secondary Objectives

- To evaluate the safety and tolerability of using AGEN1884 plus AGEN2034 plus cisplatin and gemcitabine chemotherapy in the neoadjuvant treatment of muscle-invasive bladder cancer prior to radical cystectomy,
- To estimate pathologic downstaging of ≥T2 to <T2 rate
- To investigate biological outcomes that could predict treatment efficacy

2.3 Exploratory Objectives

- To correlate clinical outcomes with immune and biologic endpoints and identify patient and tumor characteristics that can predict treatment responses

3. BACKGROUND

3.1 Muscle Invasive Bladder Cancer (MIBC)

Bladder cancer (BC) is the fourth most common malignancy in US men, with 79,030 new annual cases causing an estimated 16,780 deaths in the US in 2017. The majority of cases are superficial tumors, managed by local therapies. However, once muscle invasion is identified outcomes are much poorer, with approximately one-half of patients dying of their disease.[1] For patients with MIBC, surgery alone achieves a poor outcome, with a 5-year survival rate of 40-50%. Neoadjuvant cisplatin-based chemotherapy regimens have demonstrated a modest but absolute benefit in overall survival of 5-8% compared with surgery alone. Thus, while the standard of care in the treatment of MIBC is neoadjuvant cisplatin-based chemotherapy followed by radical cystectomy, there is a need for improvement.

3.2 Neoadjuvant Chemotherapy for MIBC

A pivotal trial randomized patients to three 28-day cycles of neoadjuvant MVAC (methotrexate, vinblastine, Adriamycin, cisplatin) plus cystectomy versus cystectomy alone, and found a 14% (p=0.06) improvement in overall survival in the combination therapy group, establishing neoadjuvant cisplatin-based chemotherapy as the standard of care.[2] The pathologic complete response (pCR) rate was 38% in the neoadjuvant MVAC arm. However, the classic MVAC regimen carried a high rate of grade 3/4 toxicity. Subsequent studies have refined the platinum containing regimen. After a study demonstrating improved toxicity with a dose-dense schedule of MVAC (ddMVAC) in metastatic urothelial cancer, two single arm phase II studies have demonstrated comparable pCR rates with neoadjuvant ddMVAC and classic MVAC. One study utilized four cycles of ddMVAC and found a pathologic response rate of 49%, with a pCR rate of 26%.[3] Another study used three cycles of ddMVAC, which yielded a pathologic response rate of 53% and a pCR rate of 38%.[4] These studies have led many experts and consensus guidelines such as the National Comprehensive Cancer Network (NCCN) to recommend a dose dense schedule of MVAC over classic MVAC.[5]

However, the multidrug MVAC regimen is not suitable for all patients, particularly the elderly bladder cancer patient with potential for cardiovascular comorbidities. Gemcitabine was found to be an active agent in advanced or metastatic urothelial cancer,[6] and a randomized phase III trial in patients with metastatic urothelial cancer comparing gemcitabine-cisplatin (GC) to classic MVAC found improved toxicity and similar efficacy with GC.[7,8] This use of GC has been extrapolated to the neoadjuvant setting, and large population based studies indicate that of those receiving neoadjuvant chemotherapy for MIBC, the most commonly used regimen is GC.[9] The NCCN also includes GC among the preferred perioperative chemotherapy regimens for MIBC.[5]

There are two primary dosing schedules for GC, a 21-day schedule and a 28-day schedule. The 21-day schedule was found to have similar dose intensity and better compliance profile than the 28-day schedule in a randomized phase II trial, so it is commonly preferred.[10]

3.3 Checkpoint Inhibition in BC

BC tumors typically carry among the highest cancer mutational loads known,[11] which correlate with the best responses to immunotherapies in other cancers.[12] Thus, the rationale for BC immunotherapy is strong, further evidenced by recent FDA approvals of two types of BC immunotherapies, antibodies blocking PD-L1 (αPD-L1) or PD-1 (αPD-1) immune checkpoint molecules.

PD-L1 is an immune checkpoint molecule that negatively regulates T cell functions through its known receptors PD-1 and CD80,[13] and is immunopathogenic in many cancers.[14] αPD-L1 has demonstrated remarkable clinical efficacy against a variety of cancers, including recent FDA-approvals of αPD-L1 for BC. PD-1 is also an immune checkpoint molecule and αPD-1 is also FDA-approved to treat BC. Despite major efficacy in a selected few patients, these immune checkpoint inhibitor antibodies are not typically curative or universally effective, with an average ~30% response rate. CTLA-4 is the third immune checkpoint molecule for which an immune checkpoint inhibitor antibody is FDA-approved (melanoma, renal cell carcinoma). While αPD-1 has significant activity as monotherapy in metastatic BC, the combination of αPD-1 and αCTLA-4 at selected doses led to even higher response rates and improvements in survival for patients with pretreated metastatic urothelial carcinoma.[15]

Combinations of two immune checkpoint inhibitors have demonstrated superior response rates to either single agent. For example, the combination of αPD-1 plus αCTLA-4 is FDA-approved for selected melanomas, as the combination provides superior response rates to either single agent.[12] The combination is also approved for the treatment of renal cell carcinoma.[16]

Likewise, αPD-1 plus αCTLA-4 provides superior response rates to either single agent to treat certain non-small cell and small cell lung cancers.[17,18]

3.4 Rationale for Combination Checkpoint Inhibition plus Chemotherapy

The most successful combination of chemotherapy with checkpoint inhibitors thus far has been in lung cancer. The KEYNOTE-021 study was a randomized phase II trial of chemotherapy-naïve advanced non-squamous, non-small cell lung cancer patients.[19] Based on phase I data showing the highest ORR with the combination of carboplatin and pemetrexed with pembrolizumab, this trial randomized patients to receive either 4 cycles of carboplatin-pemetrexed with continued pemetrexed maintenance alone or the same regimen combined with pembrolizumab 200 mg every 3 weeks for 24 months. The pembrolizumab plus chemotherapy group (n = 60) had an improved ORR of 55% (95% CI, 42-68%) compared with the chemotherapy alone group (n = 63), which had an ORR of 29% (95% CI, 18-41%). Median PFS in the combination group was 13.0 months (95% CI, 8.3-not reached) compared with 8.9 months (95% CI, 4.4-10.3) in the chemotherapy alone group. The rate of grade 3 or higher AEs was slightly higher with the combination (39%) than with chemotherapy alone (26%) but did not lead to an increased discontinuation rate (10% versus 13%, respectively). Interestingly, responses were seen regardless of tumor PD-L1 expression, with 57% ORR (95% CI, 34-79) in the PD-L1 <1% group and 54% (95% CI, 37-70) in the PD-L1 ≥1% group.

GC chemotherapy is an established standard of care in the treatment of MIBC, as detailed above. Furthermore, both agents have been shown to enhance antitumoral immune activity. Cisplatin can upregulate MHC class I expression, promote recruitment and proliferation of effector T cells, downregulate immunosuppressive elements of the tumor microenvironment, and upregulate activity of cytotoxic effectors.[20] Similarly, gemcitabine too has been shown to increase antigen presentation and MHC class I expression as well as reduce population of myeloid-derived suppressor cells *in vitro*.[21] The regimen is also most effectively administered on a 21-day schedule, similar to the investigational agents. Thus, this is an ideal chemotherapy backbone for this study of combination chemoimmunotherapy.

3.5 Rationale for pT0 as the Clinical Endpoint

Pathologic downstaging to pT0 in BC at cystectomy is strongly associated with long-term disease-free survival. For patients with pT0 at time of cystectomy (pathologic complete response or pCR), 5-year overall survival was 85%, compared to only 45% for patients with residual disease at time of cystectomy.[22] This endpoint has been used to justify dose-dense MVAC as a standard of care based on results of a phase II clinical trial, and is currently used as the primary endpoint of several ongoing neoadjuvant cystectomy trials.[4]

The reported pCR rates to cisplatin-based neoadjuvant chemotherapy vary widely, ranging from 15-41%.[4,23-26] A large, recent cross-sectional analysis of 332 patients treated at an academic institution who received neoadjuvant chemotherapy prior to cystectomy reported a pCR rate of 24.5% in the 204 patients receiving neoadjuvant GC.[26]

3.6 Product Description

3.6.1 AGEN1884

AGEN1884 is a fully human IgG1 antibody. There was no apparent pattern seen for SAEs or AEs ≥ grade 3 related to AGEN1884 or AGEN1884 dose. Most AEs were of mild-moderate severity and manageable by standard care. In Phase I trials, AGEN1884 was well tolerated in combination with the FDA-approved αPD-1 agent pembrolizumab. AGEN1884 was also well-tolerated when combined with the αPD-1 antibody AGEN2034. Its mechanisms of action, pharmacokinetic properties, AE profile and single agent efficacy are similar to FDA-approved ipilimumab (Agenus Investigator Brochure). As of ASCO 2018, AGEN1884 delivered clinical benefit in 31% of patients. A complete and durable response was observed in a patient with angiosarcoma.

3.6.2 AGEN2034

AGEN2034 is a human IgG4 antibody. In 24 evaluable subjects, up to 10 mg/kg every 2 weeks was safe and tolerable. Its safe combination with AGEN1884 is shown above. Its mechanisms of action, pharmacokinetic properties, AE profile and clinical efficacy are similar to FDA-approved anti-PD-1 agents nivolumab or pembrolizumab (Agenus Investigator Brochure). As of ASCO 2018, AGEN2034 delivered clinical benefit in 42% of patients.

3.7 Dose Rationale

Doses and schedule for AGEN1884 and AGEN2034 were based on Agenus phase I experience with these agents, with initial dosing based on FDA approval of similar anti-CTLA-4 and anti-PD-1 antibodies, respectively in melanoma. In data presented from the Phase 1/2 Study of CTLA-4 Inhibitor AGEN1884 + PD-1 Inhibitor AGEN2034 in Patients With Advanced/Refractory Solid Tumors at the European Society of Medical Oncology Annual Meeting 2018 (Abstract 1168P), 10 patients had been treated with AGEN2034 1 mg/kg q2 weeks + AGEN1884 1mg/kg q6 weeks and another 10 patients with AGEN2034 3 mg/kg q2 weeks + AGEN 1884 1 mg/kg q6 weeks. As of July 2018, no DLTs had been observed and no treatment-emergent adverse events (TEAEs) led to treatment discontinuation or death. One patient in the AGEN2034 1 mg/kg cohort had a serious TEAE considered related to treatment (diarrhea), and two patients in the AGEN2034 3 mg/kg cohort had a serious TEAE considered related to treatment (lower respiratory tract infection, pulmonary embolism). Based on these results, the phase 2 recommended dosing was determined as AGEN2034 3 mg/kg q2w + AGEN1884 1 mg/kg q6w. Preliminary clinical safety and response data are available from investigations of AGEN1884 as monotherapy [Study C-500-01], AGEN2034 as monotherapy [Study C-700-01], and AGEN1884+AGEN2034 as combination therapy [Study C-550-01]. Based on prior experience with combining chemotherapy with checkpoint inhibitors, such as from the KEYNOTE-021 study, there are not expected to be overlapping toxicities given distinct mechanisms of action.[27]

At the 01 November 2018 FDA End-of-Phase-1 (EOP1) meeting, FDA agreed to a fixed (flat) dose for AGEN2034 for Study C-750-01/Study GOG-3028. For a patient of average body weight, AGEN2034 delivered as 3 mg/kg q2w (body weight adjusted dose) or 300 mg q3w (flat dose) is expected to deliver similar AGEN2034 dose and systemic exposure over a 6-week treatment cycle. To allow for coordination with the 21-day chemotherapy backbone schedule, we have chosen the dose of AGEN2034 300 mg q3w and AGEN1884 1 mg/kg q6w. Moreover, the choice of a flat dose for AGEN2034 is expected to reduce dosing errors and reduce drug wastage.

4. INVESTIGATIONAL PLAN

4.1 Overall Study Design

This is a phase II trial to evaluate the tolerability, efficacy, and immune outcomes of AGEN1884 plus AGEN2034 concurrent with cisplatin and gemcitabine in the neoadjuvant treatment of muscle-invasive, non-metastatic bladder cancer prior to radical cystectomy. We will begin with an initial safety run-in to establish the safety of the combination prior to expansion to the full planned phase II. The overall phase II will be an open-label, single arm study in two stages to evaluate the efficacy of the combination in pathologic downstaging of MIBC. Patients will receive four 21-day cycles of neoadjuvant therapy consisting of cisplatin and gemcitabine plus AGEN2034 in all 4 cycles and AGEN1884 in cycles 1 and 3. Patients will proceed to radical cystectomy within 10 weeks after the final dose of this therapy. The primary endpoint of pathologic tumor downstaging will be assessed at the time of cystectomy.

4.1.1 Safety Run-In

The safety run-in of the study will first enroll three patients who will begin treatment with cisplatin and gemcitabine plus AGEN2034 and AGEN1884 as outlined in the treatment plan. These first 3 patients will be assessed for DLTs and there will be a pause in enrollment until all three complete the DLT period. DLT is defined from events experienced from day 1, cycle 1 through the day 1, cycle 2. If there are no DLTs in the first 3 patients, we will proceed to further accrual to stage I of phase II. If there is 1 DLT in the initial 3 patients, we will enroll 3 additional patients to the safety run-in. If $\geq$ 2 DLTs are experienced in the initial 3 patients, the study will be terminated. If there are only 2 DLTs in the 6 patients treated, further accrual can proceed. If there are >2 DLTs in the 6 patients treated, the study will be halted. All patients who receive at least two cycles of neoadjuvant therapy will be eligible for the primary efficacy endpoint of the study. Those who do not receive cystectomy or who receive <2 cycles of neoadjuvant therapy will not be evaluable for the primary efficacy endpoint and must be replaced.

4.1.2 Phase II, Stage 1

In the first stage of phase II of this study, 17 patients will be enrolled. Patients will begin treatment with cisplatin and gemcitabine plus AGEN2034 and AGEN1884 as outlined in the treatment plan. They will be evaluated with each cycle of therapy, with radiographic restaging assessment after 2 cycles of therapy and prior to the third cycle of treatment. If no disease progression is identified, patients will receive a third and fourth cycle of therapy. Following this neoadjuvant regimen, they will proceed to planned surgery following preoperative clearance within 10 weeks of the last dose of neoadjuvant therapy. Surgical specimens will be used to assess the primary endpoint of pathologic tumor downstaging to pT0. Correlative endpoints will also be measured throughout the study period as specified. The study will proceed to the second stage provided the early stopping rules have not been met and at least 5 adequately treated patients achieve a pCR. Evaluable patients are defined as those receiving at least 2 cycles of neoadjuvant immunotherapy plus chemotherapy as scheduled. Otherwise, that patient must be replaced.

4.1.3 Phase II, Stage 2

If criteria are met to continue to the second stage of the Phase II portion of the study, 19 more patients will be enrolled for a total of 36 evaluable patients. Patients will be treated and endpoints evaluated as above.

4.2 Patient Selection

4.2.1 Inclusion Criteria

1. Diagnosis of muscle-invasive, non-metastatic urothelial carcinoma of the bladder, cT2-4, N0-1, M0
2. Eligible to receive cisplatin-based chemotherapy, with eligibility defined as meeting all of the following criteria:
    a. Eastern Cooperative Oncology Group performance status of 0-1
    b. Creatinine clearance (CrCl) of $\geq$50 mL/min, as measured by 24-hour urine collection or estimated by the CKD-EPI equation. Patients with CrCl between 50 – 60 mL/min are eligible for the study but will receive split dose cisplatin
    c. Grade $\leq$ 2 hearing loss
    d. Grade $\leq$ 2 peripheral neuropathy

e. New York Heart Association Class < III heart failure

3. Eligible to receive gemcitabine as dosed here
4. Patients must have organ and marrow function meeting the criteria below:

Absolute neutrophil count	≥ 2,000/mcL
Hemoglobin	≥ 9.0 mg/mL
Platelets	≥ 100,000/mcL
Total bilirubin	within normal limits or known to be elevated due to a benign conjugation defect such as Gilbert's syndrome, as evidenced by normal conjugated bilirubin level
AST/ALT	< 3X institutional normal limits
Creatinine clearance (CrCl)	≥ 50 mL/min/1.73m2, as measured with 24 hr urine collection or estimated by CKD-EPI, whichever is greater

5. Signed, written informed consents to allow transfer of tumor tissue and production of peptides and to receive experimental treatment and monitoring if agreeable, or monitoring without experimental treatment otherwise
6. Age ≥18 years
7. Available fresh tissue from surgical excision. If fresh tissue is not available, archival tissue may be used.
8. Female subjects of childbearing potential must have a negative serum pregnancy test at screening (within 72 hours of first dose of study medication). Non-childbearing potential (other than by medical reasons) is defined as 1 of the following:
    a. ≥ 45 years of age and amenorrheic for >1 year by self-report.
    b. Amenorrheic for >2 years without a hysterectomy and oophorectomy, and follicle-stimulating hormone value in the postmenopausal range upon pretrial (screening) evaluation.
    c. Status post-hysterectomy, -oophorectomy, or –tubal ligation.

If of childbearing potential, female subjects must be willing to use adequate birth control during the study, starting with the screening visit through 120 days after the last dose of study therapy.

Male subjects with a female partner(s) of childbearing potential must agree to use a condom throughout the trial, starting with the screening visit through 120 days after the last dose of study therapy. Males with pregnant partners must agree to use a condom; no additional method of contraception is required for the pregnant partner.

Note: Abstinence is acceptable for both female and male subjects if this is the subject's established and preferred contraception method.

4.2.2. Exclusion Criteria

1. Subjects must not have previously received a checkpoint inhibitor ie, anti-PD-1, anti-PD L1, or anti CTLA-4 antibody.
2. Subjects must not have previously received anticancer medications or investigational drugs for the disease under study within the following windows:

a. ≤ 28 days for prior monoclonal antibody used for anticancer therapy, with the exception of denosumab
b. ≤ 7 days for immunosuppressive treatment for any reason, with the following exceptions:
   i. Physiologic steroid replacement for adrenal insufficiency (e.g., <10 mg prednisone per day) is permitted.
   ii. Use of inhaled or topical corticosteroid for radiographic procedures is permitted.
c. Systemic corticosteroids ≤ 7 days are not allowed except as defined above.
d. ≤ 28 days before first dose of study drug for all other investigational study drugs or devices

3. Has persisting toxicity related to prior therapy of National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (NCI-CTCAE) Grade >1 severity.
   Note: Sensory neuropathy or alopecia of Grade ≤2 is acceptable.
4. Has known severe hypersensitivity reactions to fully human monoclonal antibodies (NCI-CTCAE Version 5.0 Grade ≥3), any history of anaphylaxis, or uncontrolled asthma.
5. Active or history of any autoimmune disease (subjects with diabetes type 1, vitiligo, psoriasis, hypo- or hyperthyroid disease not requiring immunosuppressive treatment are eligible). Patients with a history of inflammatory bowel disease (including Crohn's disease and ulcerative colitis) and autoimmune disorders such as rheumatoid arthritis, systemic progressive sclerosis [scleroderma], Systemic Lupus Erythematosus or autoimmune vasculitis [e.g., Wegener's Granulomatosis] are excluded from this study.
6. Any condition requiring systemic treatment with corticosteroids (>10mg daily prednisone equivalents) or other immunosuppressive medications within 14 days prior to first dose of study drug. Inhaled steroids and adrenal replacement steroids doses >10mg daily prednisone equivalents are permitted in the absence of active autoimmune disease.
7. Uncontrolled intercurrent illness, including but not limited to uncontrolled infection, interstitial lung disease or active, non-infectious pneumonitis, symptomatic congestive heart failure, unstable angina pectoris, uncontrolled cardiac arrhythmia, or social situations that would limit compliance with study requirements in the opinion of the treating investigator or medical monitor.
8. History of intolerance or allergic reactions attributed to compounds of similar chemical or biologic composition to AGEN1884 or AGEN2034.
9. Women who are pregnant or breastfeeding.
10. Receipt of a live vaccine within 30 days prior to the first dose of study drug.
11. Inability to adhere to the protocol

4.2.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial and none will be excluded based on gender or ethnicity.

4.3 Treatment Administration

AGEN2034 with chemotherapy is always given on day 1 of eligible chemotherapy cycles and AGEN1884 is always given on day 1 of cycles 1 and 3. If chemotherapy is held for any reason, AGEN2034 and AGEN1884 are also held, to resume concurrent with chemotherapy when chemotherapy resumes. There is no specified time that chemotherapy is withheld that will exclude a patient from study, except as noted in the DLT assessment section for the safety run-in. Toxicities occurring in any of the 4 neoadjuvant cycles and 7 days thereafter with AGEN1884, AGEN2034 or cisplatin and gemcitabine are used to make toxicity assessments of these agents but not necessarily DLTs.

The order of administration will be as follows:

Premedications	Tylenol 650 mg oral, antiemetics (including dexamethasone, 5HT3 antagonist, NK1 antagonist, and olanzapine, as per investigator discretion).

AGEN2034	IV infusion (final concentration 1-2 mg/mL), over 30 minutes, after dilution in sterile, normal saline
AGEN1884	IV infusion (final concentration 1-2 mg/mL), over 30 minutes, after dilution in sterile, normal saline
Gemcitabine	IV infusion over 30 minutes
Cisplatin	IV infusion over 2 hours

Pre- and post-hydration as well as mannitol administration for cisplatin will be at investigators' discretion, but at least 2L of IV fluids must be given.

Cisplatin and gemcitabine will be prepared and administered as per standard of care. AGEN2034 and AGEN1884 will be prepared and administered as per the Agenus pharmacy manual for each agent, respectively.

4.4 Dose Modifications and Toxicity Management

Dose modifications for AGEN1884 and AGEN2034 are not permitted. Treatment may be held for toxicity as per accepted guidelines. Toxicities related to AGEN1884 and AGEN2034 are graded and managed per the SOC for toxicities related to the similar agents ipilimumab, or pembrolizumab or nivolumab, respectively, as per the well-accepted ESMO and ASCO/NCCN guidelines and as noted below.[28,29]

Dose modifications for cisplatin and gemcitabine are permitted as per standard of care. Thus, dose delays or split-dose administration of cisplatin for renal injury is permitted at investigator's discretion. Dose reduction of gemcitabine up to 25% or 750 mg/m2 is also permitted.

For patients with CrCl between 50 – 60 mL/min, cisplatin should be administered as a split dose of 35 mg/m2, either on Days 1 & 2 or Days 1 & 8 of each cycle.

Though the CKD-EPI equation may be used to determine study eligibility, the Cockroft-Gault equation will be used to determine any chemotherapy dosing as per pharmacy standard.

Recommendations for Management of Immune-Related Adverse Events (irAEs)

Immuno-oncology agents such as AGEN1884 and AGEN2034 are associated with irAEs. Early recognition and management of irAEs may mitigate severe toxicity. Investigators should also monitor subjects closely for potential irAEs, which may manifest after weeks of treatment, at the earliest. Such events may consist of persistent rash, diarrhea, colitis, autoimmune hepatitis, pneumonitis, encephalitis, arthritis, glomerulonephritis, cardiomyopathy, or uveitis and other inflammatory eye conditions.

Management algorithms have been developed to assist investigators in assessing and managing the following groups of irAEs: gastrointestinal, pulmonary, dermatological, renal, hepatic, neurological, and endocrine, among others.

AEs (both non-serious and serious) associated with drug exposure and consistent with an immune phenomenon may represent an immunologic etiology. These irAEs may be predicted based on the nature of the IMPs, its mechanism of action, and reported experience with immunotherapies that have a similar mechanism of action. An irAE can occur shortly after the first dose or several months after the last dose of treatment. Particular attention should be paid to AEs that may be suggestive of potential irAEs, as outlined below.

irAEs are considered adverse events of special interest (AESIs) and should be reported to Agenus from the time of consent to participate in this trial up to at least 90 days (90-day safety follow-up visit) from last IMP administration.

Dermatological irAEs

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue therapy per protocol.

Dermatological irAE Management Algorithm

Dermatological irAEs		
CTCAE Grade of Maculo-papular Rash	**Management**	**Follow-up**
Grade 1 Macules/papules covering <10% BSA with or without symptoms (e.g., pruritus, burning, tightness) **Grade 2** Macules/papules covering 10-30% BSA with or without symptoms (e.g., pruritus, burning, tightness); limiting instrumental ADL; rash covering > 30% BSA with or without mild symptoms	Symptomatic therapy (e.g., antihistamines, topical corticosteroids). Continue AGEN1884 and/or AGEN2034 therapy per protocol.	**If persists >1 to 2 weeks or recurs:** Consider skin biopsy. Delay AGEN1884 and/or AGEN2034 therapy. Consider 0.5-1 mg/kg/day methylprednisolone IV or oral equivalent. Once improving, taper corticosteroids over ≥1 month; consider prophylactic antibiotics for opportunistic infections; and resume AGEN1884 therapy per protocol. **If worsens:** Treat as Grade 3-4.
Grade 3 Macules/papules covering >30% BSA with moderate or severe symptoms; limiting self-care ADL	Delay or discontinue AGEN1884 and/or AGEN2034 therapy per protocol. Consider skin biopsy. Dermatology consult 1-2 mg/kg/day methylprednisolone IV or IV equivalent.	**If improves to Grade 1:** Taper corticosteroids over ≥1 month; add prophylactic antibiotics for opportunistic infections. Resume AGEN1884 and/or AGEN2034 therapy per protocol (unless AGEN1884 and/or AGEN2034 was discontinued [vs. delayed] for the event).

Abbreviations: ADL = activities of daily living; BSA = body surface area; CTCAE = Common Terminology Criteria for Adverse Events; irAE = immune-related adverse event; IV = intravenous.

Gastrointestinal irAEs

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis

Gastrointestinal irAEs Management Algorithm

Gastrointestinal irAEs		
CTCAE Grade of Diarrhea/Colitis	**Management**	**Follow-up**
Grade 1 Diarrhea: Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline Colitis:	Continue AGEN1884 and/or AGEN2034 therapy per protocol. Symptomatic treatment.	Close monitoring for worsening symptoms. Educate subject to report worsening immediately. Consider symptomatic treatment including hydration, electrolyte replacement, dietary changes (e.g.,

Gastrointestinal irAEs		
CTCAE Grade of Diarrhea/Colitis	**Management**	**Follow-up**
Asymptomatic; clinical or diagnostic observations only; intervention not indicated		American Dietetic Association colitis diet), and loperamide. **If worsens:** Treat as Grade 2 or 3-4.
Grade 2 Diarrhea: Increase of 4-6 stools per day over baseline; moderate increase in ostomy output compared to baseline; limiting instrumental ADL Colitis: Abdominal pain; mucus or blood in stool	Delay AGEN1884 and/or AGEN2034 therapy per protocol. Consider prompt diagnostic work-out and early start of corticosteroids	**If improves to Grade 1:** Resume AGEN1884 and/or AGEN2034 therapy per protocol. **If persists >5 to 7 days or recurs:** 0.5-1 mg/kg/day methylprednisolone or equivalent. Consider infliximab administration if no clear early response to corticosteroids. When symptoms improve to Grade 1, taper corticosteroids over ≥1 month; consider prophylactic antibiotics for opportunistic infections; resume AGEN1884 and/or AGEN2034 therapy per protocol. **If worsens or persists >3 to 5 days with oral corticosteroids:** Treat as Grade 3-4.
Grade 3 Diarrhea: Increase of ≥7 stools per day over baseline; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self-care ADL Colitis: Severe abdominal pain; peritoneal signs **Grade 4** Diarrhea: Life-threatening consequences; urgent intervention indicated Colitis: Life-threatening consequences; urgent intervention indicated	Discontinue AGEN1884 and/or AGEN2034 therapy per protocol. 1-2 mg/kg/day methylprednisolone IV or equivalent. Add prophylactic antibiotics for opportunistic infections. Consider lower endoscopy.	**If improves:** Continue corticosteroids until Grade 1, then taper over ≥1 month. **If persists >3 to 5 days or earlier if no clear response or recurs after improvement:** Add infliximab 5 mg/kg (if no contraindication). **Note:** Infliximab should not be used in cases of perforation or sepsis.

Abbreviations: ADL = activities of daily living; CTCAE = Common Terminology Criteria for Adverse Events; irAE = immune-related adverse event; IV = intravenous.

Pulmonary irAEs

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue therapy per protocol. Evaluate with imaging and pulmonary consultation

Pulmonary irAE Management Algorithm

Pulmonary irAEs		
CTCAE Grade of Pneumonitis	**Management**	**Follow-up**
Grade 1 Radiographic changes only **Grade 1** Asymptomatic; clinical or diagnostic observations only, including radiographic changes	Consider delay of AGEN1884 and/or AGEN2034 therapy per protocol. Monitor for symptoms every 2-3 days. Consider pulmonary and infectious disease consults.	Re-image every ≥3 weeks. **If worsens:** Treat as Grade 2 or Grade 3-4.
Grade 2 Symptomatic; medical intervention indicated; limiting instrumental ADL	Delay AGEN1884 and/or AGEN2034 therapy per protocol. Pulmonary and infectious disease consults. Monitor symptoms daily; consider hospitalization. 1 mg/kg/day methylprednisolone IV or oral equivalent. Consider bronchoscopy, lung biopsy.	Re-image every 1-3 days. **If improves:** When symptoms return to near baseline, taper corticosteroids over ≥1 month, then resume AGEN1884 and/or AGEN2034 therapy per protocol and consider prophylactic antibiotics. **If not improving after 2 weeks or worsening:** Treat as Grade 3-4.
Grade 3 Severe symptoms; limiting self-care ADL; oxygen indicated **Grade 4** Life-threatening respiratory compromise; urgent intervention indicated (e.g., tracheotomy or intubation)	Discontinue AGEN1884 and/or AGEN2034 therapy per protocol. Hospitalize. Pulmonary and infectious disease consults. 2-4 mg/kg/day methylprednisolone IV or IV equivalent. Add prophylactic antibiotics for opportunistic infections. Consider bronchoscopy, lung biopsy.	**If improves to baseline:** Taper corticosteroids over ≥6 weeks. **If not improving after 48 hours or worsening:** Add additional immunosuppression (e.g., infliximab, cyclophosphamide, IV immunoglobulin, mycophenolate mofetil).

Abbreviations: ADL = activities of daily living; CTCAE = Common Terminology Criteria for Adverse Events; irAE = immune-related adverse event; IV = intravenous.

Hepatic irAEs

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue therapy per protocol. Consider imaging for obstruction

Hepatic irAE Management Algorithm

Hepatic irAEs		
CTCAE Grade of Liver Test Elevation	**Management**	**Follow-up**
Grade 1 **ALT/AST** >ULN - 3.0 × IULN if baseline was normal; 1.5-3.0 × baseline if baseline was abnormal **Bilirubin** >ULN - 1.5 × IULN if baseline was normal; > 1.0-1.5 × baseline if baseline was abnormal	Continue AGEN1884 and/or AGEN2034 therapy per protocol.	Continue LFT monitoring per protocol. **If worsens:** Treat as Grade 2 or Grade 3-4.
Grade 2 **ALT/AST** >3.0-5.0 × IULN if baseline was normal; >3.0- 5.0 × baseline if baseline was abnormal **Bilirubin** >1.5-3.0 × IULN if baseline was normal; >1.5-3.0 × baseline if baseline was abnormal	Delay AGEN1884 and AGEN2034 therapy per protocol. Increase frequency of monitoring to every 3 days. If subject has concurrent AST or ALT >3 × IULN and total bilirubin >2 × IULN, discontinue AGEN1884 therapy per protocol.	**If returns to baseline:** Resume routine monitoring; resume AGEN1884 and AGEN2034 therapy per protocol. **If elevations persist >5 to 7 days or worsen:** 0.5-1 mg/kg/day methylprednisolone IV or oral equivalent. When LFT returns to Grade 1 or baseline, taper corticosteroids over ≥1 month, consider prophylactic antibiotics for opportunistic infections, and resume AGEN1884 and/or AGEN2034 therapy per protocol.
Grade 3 **ALT/AST** >5.0-20.0 × IULN if baseline was normal; >5.0-20.0 × baseline if baseline was abnormal **Bilirubin** >3.0-10.0 × IULN if baseline was normal; >3.0-10.0 × baseline if baseline was abnormal **Grade 4** **ALT/AST** >20.0 × IULN if baseline was normal; >20.0 × baseline if baseline was abnormal **Bilirubin** >10.0 × IULN if baseline was normal; >10.0 × baseline if baseline was abnormal	Discontinue AGEN1884 and/or AGEN2034 therapy per protocol. Increase frequency of monitoring to every 1-2 days. 1-2 mg/kg/day methylprednisolone IV or oral equivalent.* Add prophylactic antibiotics for opportunistic infections. Consult gastroenterology. Consider obtaining MRI/CT scan of liver and liver biopsy if clinically warranted.	**If returns to Grade 2:** Taper corticosteroids over ≥1 month. **If does not improve in >3 to 5 days, worsens or rebounds:** Add mycophenolate mofetil 1 g twice daily. If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines.

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; CT = computed tomography; CTCAE = Common Terminology Criteria for Adverse Events; IULN = Institutional upper limit of institutional normal; irAE = immune-related adverse event; IV = intravenous; LFT = liver function test; MRI = magnetic resonance imaging; ULN = upper limit of normal.
* The recommended starting dose for Grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Endocrine irAEs

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and

Endocrine irAE Management Algorithm

Endocrine irAEs		
Endocrine Disorder	**Management**	**Follow-up**
Asymptomatic TSH abnormality	Continue AGEN1884 and/or AGEN2034 therapy per protocol. **If TSH <0.5 × lower limit of normal (LLN) or TSH >2 × IULN, or consistently out of range in 2 subsequent measurements:** Include free thyroxine at subsequent cycles as clinically indicated; consider endocrinology consult.	
Symptomatic endocrinopathy	Evaluate endocrine function. Consider pituitary scan. **Symptomatic with abnormal laboratory tests and/or pituitary scan**: Delay AGEN1884 and/or AGEN2034 treatment per protocol; 1-2 mg/kg/day methylprednisolone IV or oral equivalent; initiate appropriate hormone therapy. **No abnormal laboratory tests and/or pituitary MRI scan but symptoms persist**: Repeat laboratory tests in 1-3 weeks, MRI in 1 month. Suspected thyroid dysfunction based on clinical symptoms. Check TSH and T4/T3. Low TSH and evaluated T4/T3 thyrotoxicosis likely due to thyroiditis 1. Beta-blockers for	**If improves (with or without hormone replacement):** Taper corticosteroids over ≥1 month and consider prophylactic antibiotics for opportunistic infections. Resume AGEN1884 and/or AGEN2034 therapy per protocol. Subjects with adrenal insufficiency may need to continue corticosteroids with mineralocorticoid component.

Endocrine irAEs		
Endocrine Disorder	**Management**	**Follow-up**
	symptomatic patients 2. Hold immunotherapy when CTCAE garde >=3 3. Restart therapy when CTCAE grade <=2 4. Check thyroid functions every 2-3 weeks 5. If Graves disease, treat with methimazole or RAI/ surgery Once thyroid function test shows elevated TSH and low T4, diagnosis of hypothyroidism is made Elevated TSH, low T4/T3 primary hypothyroidism 1. Treat with levothyroxine 2. Monitor TFTs every 6-8 weeks until stable, then 6-12 monthly Elevated TSH, low T4/T3 primary hypothyroidism or low TSH, low T4/T3 central hypothyroidism 1. Treat with levothyroxine 2. Monitor TFTs every 6-8 weeks until stable, then 6-12 monthly	
Suspicion of adrenal crisis (e.g., severe dehydration, hypotension, shock out of proportion to current illness)	Delay or discontinue AGEN1884 and/or AGEN2034 therapy per protocol. Rule out sepsis. Stress dose of IV corticosteroids with mineralocorticoid activity. IV fluids. Consult endocrinologist. If adrenal crisis ruled out, treat as above for symptomatic endocrinopathy.	

Abbreviations: irAE = immune-related adverse event; IULN = institutional upper limit of normal; IV = intravenous; LLN = lower limit of normal; MRI = magnetic resonance imaging; TSH = thyroid-stimulating hormone.

Renal irAEs

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue therapy per protocol

Renal irAE Management Algorithm

Renal irAEs		
CTCAE Grade of Creatinine Increased	**Management**	**Follow-up**
Grade 1 >IULN - 1.5 × IULN	Continue AGEN1884 and/or AGEN2034 therapy per protocol. Monitor creatinine weekly.	**If returns to baseline:** Resume routine creatinine monitoring per protocol. **If worsens:** Treat as Grade 2 or Grade 3-4.
Grade 2 >1.5-3.0 × baseline; >1.5-3.0 × IULN **Grade 3** >3.0 × baseline; >3.0-6.0 × IULN	Delay AGEN1884 and/or AGEN2034 therapy per protocol. Monitor creatinine every 2-3 days. 0.5-1.0 mg/kg/day methylprednisolone IV or oral equivalent. Consider renal biopsy.	**If returns to Grade 1:** Taper corticosteroids over ≥1 month, consider prophylactic antibiotics for opportunistic infections, and resume AGEN1884 and/or AGEN2034 therapy and routine creatinine monitoring per protocol. **If elevations persist > 7 days or worsen:** Treat as Grade 4.
Grade 4 Creatinine >6 × IULN	Discontinue AGEN1884 and/or AGEN2034 therapy per protocol. Monitor creatinine daily. 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent. Consult nephrologist. Consider renal biopsy.	**If returns to Grade 1:** Taper corticosteroids over ≥1 month and add prophylactic antibiotics for opportunistic infections.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; irAE = immune-related adverse event; IULN = institutional upper limit of normal; IV = intravenous.

Neurological irAEs

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue therapy per protocol

Neurological irAE Management Algorithm

Neurological irAEs		
CTCAE Grade of Nervous System Disorder, Other	**Management**	**Follow-up**
Grade 1 Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Continue AGEN1884 and/or AGEN2034 therapy per protocol.	Continue to monitor subject. **If worsens:** Treat as Grade 2 or Grade 3 4.
Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL	Delay AGEN1884 and/or AGEN2034 therapy per protocol. Treat symptoms per local guidelines. Consider 0.5-1.0 mg/kg/day methylprednisolone IV or oral equivalent.	**If returns to baseline:** Resume AGEN1884 therapy per protocol. **If worsens:** Treat as Grade 3-4.
Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; limiting self-care ADL **Grade 4** Life-threatening consequences; urgent intervention indicated	Discontinue AGEN1884 and/or AGEN2034 therapy per protocol. Obtain neurology consult. Treat symptoms per local guidelines. 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent. Add prophylactic antibiotics for opportunistic infections.	**If improves to Grade 2:** Taper corticosteroids over ≥1 month. **If worsens or atypical presentation:** Consider IV immunoglobulin or other immunosuppressive therapies per local guidelines.

4.5 Study Safety Review

4.5.1 Study Safety Review Committee

The study safety review committee consists of the Mays Cancer Center DSMB and the principal investigator or designated research staff treating subjects on study. Safety will be monitored with biweekly study team calls and reviewed during safety committee meetings.

4.5.2 Dose-Limiting Toxicities

For the purposes of safety evaluation during the safety run-in portion of the study, subjects will be monitored for DLTs during the first cycle of chemotherapy plus immunotherapy agents. However, should AEs meeting DLT criteria occur outside this DLT evaluation period, the safety review committee will evaluate alternative doses or administration methods if warranted. Any subject who develops a DLT will be removed from study treatment and followed for safety. The subject once removed from study treatment may proceed to radical cystectomy, in which case he/she would remain evaluable for the primary endpoint if he/she meets evaluability criteria as set forth.

4.5.2.1 Definition of Dose Limiting Toxicity

DLT, as defined below, will be evaluated throughout concurrent therapy with cisplatin, gemcitabine, AGEN1884 and AGEN2034. Toxicity that is clearly and directly related to the primary disease or to another etiology is excluded from this definition. The following drug related AEs will be DLTs:

Any grade 5 AEs

Immune Related AEs (irAEs)

1. Grade 4 immune-related AEs
2. Any grade 3 irAE that does not resolve to ≤ Grade 1 or to baseline with immunosuppressive therapy within 3 weeks of its onset
3. Any grade 3 central nervous system (CNS)-related irAE regardless of duration, or reversibility

Any Grade 4 non-hematologic, non-laboratory AEs will be considered DLTs with the exception of isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset

Grade 4 hematologic AEs with following exceptions:

1. Grade 4 neutropenia that is not associated with fever or systemic infection that improves by at least 1 grade within 3 days. Grade 4 febrile neutropenia will be a DLT regardless of duration or reversibility.
2. Grade 4 lymphopenia
3. Grade 4 thrombocytopenia that is not associated with clinically significant bleeding that requires medical intervention, and improves by at least 1 grade within 3 days
4. Other Grade 4 hematologic AEs that last less than 7 days

The definition of DLT excludes the following other conditions:

1. Grade 3 fatigue lasting ≤ 7 days
2. Grade 3 endocrine disorder (thyroid, pituitary, and/or adrenal insufficiency) that is managed with or without systemic corticosteroid therapy and/or hormone replacement therapy and the subject is asymptomatic
3. Grade 3 infusion-related reaction (first occurrence and in the absence of steroid prophylaxis) that resolves within 6 hours with appropriate clinical management

4. Grade 3 flu-like symptoms or fever lasting ≤6 hours, which are controlled with medical management

Immune-related AEs are defined as AEs of an immune nature (*i.e.,* inflammatory) in the absence of a clear alternative etiology. In the absence of a clinically significant abnormality, repeat laboratory testing will be conducted to confirm significant laboratory findings prior to designation as a DLT.

All DLTs will be assessed by the investigator using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

4.5.2.2 Stopping Early Rules for Enrollment Based on Toxicities

DLTs for either investigational agent tested will stop the protocol. If any of the investigational agents cannot be continued, the patient will come off all study treatment and proceed as per standard of care.

In addition, the Mays Cancer Center DSMB 2 in conjunction with the PI will review the safety of all study subjects. Should any AEs be consistent with DLTs (*e.g.*, from immune-related toxicities), they will be evaluated for relatedness to study agents. Should AEs meeting DLT criteria occur beyond the defined DLT evaluation period, the DSMB should be notified by the PI of the study to evaluate alternative doses or administration methods.

If there is a subject death within 30 days of any study drug administration for reasons not related to the subject's disease recurrence, the trial will be put on hold to accrual and treatment will be held for subjects on study until an appropriate evaluation of the cause of death is determined, unless the death is from accidental trauma. If the PI determines that the death is related to study treatment, a study amendment and/or other corrective action plan will be established. If the death is determined not to be related to study treatment, the study will re-open to accrual and subjects on study will resume treatment.

4.5.2.3 Prior and Other Concomitant Therapy

Allowed and disallowed prior and concomitant therapies are described under inclusion and exclusion criteria. Investigators are permitted to use any SOC agents to mitigate any adverse events related to SOC chemotherapy, and any SOC agents used to mitigate adverse events related to nivolumab or pembrolizumab, or ipilimumab for AGEN2034 or AGEN1884 adverse events, respectively. The principal investigator has ultimate discretion to grant exceptions to the use of otherwise excluded agents in mitigation of adverse events.

4.6 Study Schedule

Screening Visit

This will occur within 30 days of study enrollment.

Pretreatment: Week -1 to 0

All final clinical assessments are made and initial screening laboratory and imaging studies are accomplished and recorded. The treatment consent is signed. Baseline imaging and laboratory studies must be accomplished within 45 days of the first treatment on this protocol. Blood specimens will be drawn for the research repository.

Treatment and Evaluation Period: Weeks 1 to 15

Subjects receive up to 4 cycles of neoadjuvant cisplatin-gemcitabine chemotherapy plus AGEN2304 and AGEN1884, which takes around 12 weeks. Imaging is done after 2 cycles of therapy, prior to cycle 3, to rule out radiographic progression, as is SOC. After completion of the planned 4 cycles of neoadjuvant therapy, subjects will proceed to radical cystectomy with an interval of no greater than 10 weeks from the last dose of neoadjuvant therapy. The surgical pathology specimens will then be used to evaluate for the primary outcome of pathologic tumor downstaging.

Research phlebotomies will be performed at baseline and on day 1 of cycle 3 at time of cystectomy, and at the first visit in the follow-up timeframe.

Follow-up Period: Weeks 15 to 52

Subjects will be followed every 3 weeks during experimental therapies (typically being seen at treatment cycles), then every 3 months after the end of treatment visit for 1 year from the initiation of experimental treatments, and then quarterly but no less than for 1 year from initiation of treatment. Blood specimens and urine collection will be drawn for the repository during the first 3-month follow-up.

End-of-Treatment Visit

Survival Follow-up

Patients are followed in clinic through year 1 after treatment as defined above. Thereafter, they will be seen based on their clinical stability but no less than every 6 months for a total of 2 years. After 1 year, a telephone call can be substituted for a clinic visit for stable patients.

4.7 Demographics and Screening Procedures

Demographics and Medical/Cancer History. These are recorded in a HIPAA compliant manner per Mays Cancer Center protocols.

Eastern Cooperative Oncology Group Performance Status. Patients must have an ECOG performance status of 0-1 within the screening period to be eligible.

4.8 Correlative Biology

The goal of this repository is to capture immune profiles of genitourinary cancer patients and tumors using whole blood, fat, urine, and tissue. These immune profiles will be critical to understanding the importance of immunity in regulating cancer, including a specific project to determine the ability of the AGEN1884 and AGEN2034 to modulate immune profiles. The specimens will also be stored for potential use in additional studies in the future. Currently our focus is largely related to bladder cancer.

Studies of blood material

Blood specimens will be banked for future research. Blood samples (30mLs at each visit) will drawn at the time points listed in the Study Calendar.

Studies of tumor material

Surgical tumor specimens remaining from original TURBT and radical cystectomy banked for future research.

4.9 Disease Assessments

4.9.1 Imaging

Imaging is done using standard equipment and read by a radiologist. CT, MRI or CT/PET fusion are acceptable.

4.9.2 Pathologic Response Criterion

The primary efficacy assessment will be pCR rate (defined as no residual tumor in bladder and lymph nodes on resected specimen). Surgery should be performed within 6 weeks after completing up to 4 cycles (last dose) of neoadjuvant therapy, but can be done up to 10 weeks after treatment ends to be evaluable. Otherwise this patient must be replaced for response evaluation. Pelvic lymph node dissection

should include a minimum standard lymph node dissection, which includes bilateral obturator, external iliac, and internal iliac lymph nodes. For histologic assessment, all tumor and lymph node tissue should be sectioned at 0.5 centimeter intervals. The specimen should be sampled as follows: at least 1 section per centimeter of primary tumor, or all tumor if less than 5 cm, and entirety of all lymph nodes submitted. The percentage of viable tumor cells in sections containing tumor should be evaluated. For assessments of pathological response, at least 1 section per centimeter of the tumor should be evaluated for all tumor and lymph node samples. A minimum of 5 slides should be evaluated, even if tumor diameter is < 5cm.

4.10 Safety Assessments

Vital Signs are aural temperature, respiratory rate, peripheral pulse and blood pressure.

Physical Examination is done per SOC.

Clinical Laboratory Assessments and Urinalysis are done per SOC.

Pregnancy Test is per SOC.

Concomitant Medications are recorded by study personnel.

Disease Recurrence, Secondary Cancer, Elective Surgery, and Death are recorded per routine Mays Cancer Center routine procedures.

Table 2, Study Calendar

	Baseline	C1D1 (Day 1)	C1D8 (Day 8 ±2 days)	C2D1 (Day 22 ±2 days)	C2D8 (Day 29 ±2 days)	C3D1 (Day 43 ±2 days)	C3D8 (Day 50 ±2 days)	C4D1 (Day 64 ±2 days)	C4D8 (Day 71 ±2 days)	End of Treatment (Day 85 ±5 days)	Cystectomy (within 10 weeks of last chemo)	Follow Up (q3 months post-Cystectomy) +/- 7 days
AGEN1884 Infusion		X				X						
AGEN2034 Infusion		X		X		X		X				
Cisplatin 70mg/m2 Infusion*		X		X		X		X				
Gemcitabine 1000mg/m2 Infusion		X	X	X	X	X	X	X	X			
History & Physical	X	X		X		X		X		X		X
Vital Signs	X	X	X	X	X	X	X	X	X	X		
ECOG	X	X		X		X		X		X		
NYHA	X											
Informed Consent	X											
CBC, CMP	X	X	X	X	X	X	X	X	X	X		
TSH	X	X		X		X		X		X		
AM cortisol	X											
Urine collection	X					X					X	X^
24 hr urine CrCl	X											
Imaging (CT Chest and CT or MRI A/P)	X					X				X		
Serum Pregnancy Test	X	X		X		X		X				

	Baseline	C1D1 (Day 1)	C1D8 (Day 8)	C2D1 (Day 22)	C2D8 (Day 29)	C3D1 (Day 43)	C3D8 (Day 50)	C4D1 (Day 64)	C4D8 (Day 71)	End of Treatment	Cystectomy (within 10 weeks of last chemo)	Follow Up (q3 months post-EOT) +/- 7 days
Adverse Events	X	X	X	X	X	X	X	X	X	X		X
Survival Assessment												X#
Tumor pathologic assessment	X										X	
Concurrent medication review	X	X		X		X		X		X		
Tumor tissue collection (archival or fresh)	X										X	
Research Blood draw	X					X					X	X^

^ denotes sample is only required at first of the 3-month follow up visits

# denotes follow up to occur every 3 months for first year then as per section 4.6

Note: Imaging performed after treatment has been initiated will be done after cycle 2 is administered and before cycle 3.

5. STATISTICAL CONSIDERATIONS

5.1 Study Design/Endpoints

Primary Endpoint(s):

- pT0 or pCR, defined as eradication of all tumor from resected bladder and lymph node tissue, following neoadjuvant chemotherapy

Secondary Endpoint(s):

- Toxicity per CTCAE v5.0
- Pathologic downstaging to <T2
- Completion of surgery
- Progression-free survival at 1 year

Exploratory Endpoint(s):

- Correlative immune outcomes

The study begins with enrolment of 3 patients who will be followed for DLTs that occur within the first cycle of treatment. If no patients experience a DLT during the window, further accrual as per the Simon two-stage design below will proceed. If one DLT is observed in the first 3 patients during the window, an additional 3 patients will be enrolled. If $\leq$2 DLTs occur in the first 6 patients during the window, further accrual to the study will proceed. If >2 DLTs occur in the first 6 patients during the window, the study results will be assessed if substantial modifications to the protocol would be required.

For the overall study, we will utilize a modified Simon two-stage design employing a minmax design, enrolling 17 patients in stage I and subsequently 19 more patients in stage II if early termination criteria are not met. There are early termination criteria for futility. To be evaluable for the primary endpoint, patients must have received at least two cycles of neoadjuvant therapy and undergone cystectomy. Using a historical pCR rate of 0.25 as the null hypothesis, we will seek to demonstrate a rate of 0.45. The reported pCR rates to cisplatin-based neoadjuvant chemotherapy vary widely, ranging from 15-41%[4,23-26]. We have chosen a rate of 25% based on the pCR rate for GC from a large, recent cross-sectional analysis of 332 patients who received neoadjuvant chemotherapy prior to cystectomy.[26] We will submit 17 patients to treatment and fail to reject the null hypothesis and terminate the study unless at least 5/17 pCRs are observed. If at least 5 pCRs are observed, we will submit an additional 19 patients to treatment. If at least 14/36 pCRs are observed we will reject the null hypothesis in favor of the sought improvement in success rate. This modified Simon two-stage design yields a type I error rate of 0.044 and power of 0.8 when the true response rate is 0.45.

Of note, if the therapy is found not to meet criteria for continuation to Stage 2 of the Simon two-stage design based on pCR rate, but vastly improves the downstaging to <pT2 rate, a discussion will be conducted among investigators and the sponsor to consider amending the protocol to continue enrollment with an alternate primary endpoint of <pT2 rate. The advantages and disadvantages of such an amendment, along with ethical concerns would be addressed prior to re-opening the study.

The number screened, the number of screen failures by reason, the number enrolled, and the number lost to follow-up reason will be tabulated. The Simon two-stage design results will be descriptively summarized. Secondary end points will be descriptively summarized. Progression free survival will be described with a graphic of the Kaplan-Meier curve, the median time to progression, and a 95% confidence interval for the median time to progression. Tabular summaries, graphics, and the median time to progression will be provided with R[30].

6. DATA COLLECTION

All quantitative study data will be entered into Research Electronic Data Capture (REDCap), a secure web-based database, which the research staff is well trained to use and has been studied for many other clinical trials.

7. DATA REPORTING / REGULATORY REQUIREMENTS

Data and Safety Monitoring Oversight

A Data and Safety Monitoring Plan is required for all individual protocols conducted at MAYS CANCER CENTER. All protocols conducted at MAYS CANCER CENTER are covered under the auspices of the MAYS CANCER CENTER Institutional Data Safety Monitoring Plan (DSMP).

The MAYS CANCER CENTER Institutional DSMP global policies provide individual trials with:

- institutional policies and procedures for institutional data safety and monitoring,
- an institutional guide to follow,
- monitoring of protocol accrual by the MAYS CANCER CENTER Protocol Review Committee,
- review of study forms and orders by the Forms Committee,
- tools for monitoring safety events,
- independent monitoring and source data verification by the MAYS CANCER CENTER QA Monitor/Auditor
- monitoring of UPIRSO's by the Director of Quality Assurance and DSMC,
- determining level of risk (Priority of Audit Level Score – PALS),
- oversight by the Data Safety Monitoring Committee (DSMC), and
- verification of protocol adherence via annual audit for all Investigator Initiated Studies by the MAYS CANCER CENTER Quality Assurance Division.

<u>Monitoring Progress and Safety</u>

Due to the risks associated with participation in this protocol, the MAYS CANCER CENTER DSMB in conjunction with the Principal Investigator will perform assessment of adverse events, adverse event trends and treatment effects on this study. The MAYS CANCER CENTER DSMB acts as an independent Data Safety Monitoring Board (DSMB) for IIS conducted at MAYS CANCER CENTER. The MAYS CANCER CENTER DSMB will monitor data throughout the duration of a study to determine if continuation of the study is appropriate scientifically and ethically. An additional layer of review is provided by the MAYS CANCER CENTER Data Safety Monitoring Committee (DSMC) who will review DSMB quarterly reports.

Baseline events and adverse events will be captured using the MAYS CANCER CENTER Master Adverse Events Document for each patient using CTCAE V. 5.0 for the grading and attribution of adverse events. Usage of the MAYS CANCER CENTER Master Adverse Events Document centrally documents:

- the event and grades the seriousness of the event,
- if the event was a change from baseline,
- the determination of the relationship between the event and study intervention,
- if the event was part of the normal disease process, and
- what actions were taken as a result of the event.

Safety Definitions:
For this study, the following safety definitions will be applicable:

Adverse Event Definition: An adverse event (AE) is defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. For this study, all adverse events will be documented starting with administration of the first investigational agent.

Serious Adverse Event Definition: is any adverse event that:

1. results in death;
2. is life-threatening (places the subject at immediate risk of death from the event as it occurred);
3. results in inpatient hospitalization or prolongation of existing hospitalization;
4. results in a persistent or significant disability/incapacity;
5. results in a congenital anomaly/birth defect; or
6. based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition

Unanticipated Problems Involving Risks to Subjects or Others Definition: Unanticipated problem involving risk to subjects or others includes any incident, experience or outcome that meets all of the following criteria:

A. unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied (note: the unfounded classification of a serious adverse event as "anticipated" constitutes serious non-compliance);
B. definitely related or probably related to participation in the research; and
C. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized

Reporting Requirements
For this study, the Master Adverse Events Documents collected on patients for this protocol will be reviewed by the Principal Investigator to determine if a serious safety problem has emerged that result in a change or early termination of a protocol such as:

- dose modification,
- suspending enrollment due to safety or efficacy, or
- termination of the study due to a significant change in risks or benefits.

The PI will provide the DSMB with a summary of adverse events and serious adverse events on a quarterly basis for discussion and review during their meetings. The first report will be due to the DSMB the quarter immediately following enrollment of the first subject. The DSMB meets in March, June, September and December, however will additionally review events and issues on an as needed basis.

The PI (or designee) is responsible for notifying sub-investigators regarding DSMB recommendations to modify the dosage (dose escalate, dose decrease) or expand the dosage to ensure that subsequent subjects are treated at the currently approved dose level. Changes to dosing should be approved by the DSMB

prior to proceeding. To further ensure safety, the study coordinator will notify the Investigative Drug Section and confer with the study PI on Cycle 1 Day 1 dosing orders to ensure the study drug dose escalations or de-escalation have has been updated correctly.

Dose modifications for AGEN1884 and/or AGEN2034 are as set forth in dose escalation/de-escalation rules above.

Toxicities related to AGEN1884 and AGEN2034 are managed per the SOC for toxicities related to the similar agents ipilimumab and pembrolizumab, respectively. Management guidelines are published by ASCO/NCCN.[29]

For SOC chemotherapy, toxicities, dose adjustments and treatment delays are managed at investigator discretion, consistent with the guidelines for SOC.

Discontinuation of Therapy
Treatment with specific investigational agents should be discontinued if 1 or more of the following occur:

- DLT occurrence or other unacceptable study drug–related AE (based on the investigator's clinical decision that the subject is unable to tolerate therapy).
- Recurrence of disease
- Study discontinuation criterion met
- Investigator decision
- Subject nonadherence to protocol

Subjects who discontinue therapy prior to recurrence will continue to undergo complete disease assessments according to SOC for up to 1 year after first study drug administration, or until a criterion for discontinuation of disease assessment is met. If subjects are clinically stable at 6 months, imaging frequency will be decreased to every 12 weeks until 1 year. Subjects will be followed for safety for ≥30 days following the last administration of investigational agents.

Discontinuation from Disease Assessment
Subjects will be discontinued from disease evaluation if 1 or more of the following occur:

- Recurrence of disease
- Study discontinuation criterion met
- Nonadherence with protocol.

Discontinuation from Study
Subjects will be discontinued from the study for 1 or more of the following:

- Withdrawal of consent
- Loss to follow-up
- Death
- Subject nonadherence.
- Completion of full study period
- Administrative study closure

Premature Discontinuation of the Trial
The entire trial may be discontinued prematurely in the event of any of the following:

- New information leading to unfavorable risk-benefit judgement of the study treatment due to (*e.g.*):
  - Occurrence of significant adverse reactions.
  - Other unfavorable safety findings. **Note**: Evidence of inefficacy may arise from this trial or from other trials, and unfavorable safety findings may arise from clinical or non-clinical examinations (*e.g.*, toxicology).
- Sponsor's decision that continuation of the trial is unjustifiable for medical or ethical reasons
- Poor enrollment of subjects, making completion of the trial within an acceptable timeframe unlikely
- Discontinuation of development of the study treatment

Health authorities and Independent Ethics Committees (IECs)/Institutional Review Boards (IRBs) will be informed about trial discontinuation in accordance with applicable regulations.
The entire trial may be terminated or suspended upon request of health authorities and/or by the DSMB.

As per the MAYS CANCER CENTER DSMP, any protocol modifications, problematic safety reports, unanticipated problems, and suspension or early termination of a trial must be reported to the DSMB and all members of the research team. Furthermore, the PI of this study will promptly notify all study affiliates, the UT HEALTH IRB, the MAYS CANCER CENTER DSMB, and the FDA via a FDA Form 3500Aa written IND safety report of any adverse events that are either serious and/or unexpected. Suspension and early termination of a trial must also be reported immediately to the Director of Quality Assurance who will promptly notify the UT HEALTH IRB.

The PI will review the Master Adverse Events documents to determine the significance of the reported events and will provide findings using the Investigator Initiated Study Quarterly DSMB Report Form on a quarterly basis with the DSMB. The DSMB will review the information provided by the PI and report to the MAYS CANCER CENTER DSMB on a quarterly basis, unless an emergent issue has been identified. The Investigator Initiated Study Quarterly DSMB Report Form includes information on adverse events, current dose levels, number of patients enrolled, significant toxicities per the protocol, patient status (morbidity and mortality) dose adjustments with observed response, and any interim findings. Any trend consisting of three or more of the same event will be reported to the MAYS CANCER CENTER DSMB for independent review outside of the quarterly reporting cycle, which begins the quarter after the first patient is enrolled on the protocol. The DSMB will also provide its findings to the MAYS CANCER CENTER's Regulatory Affairs Division so that it may be provided to the UT HEALTH IRB with the protocol's annual progress report. Conflict of interest is avoided by the independent reviews of the MAYS CANCER CENTER DSMB, MAYS CANCER CENTER DSMC, and by ongoing independent review of UPIRSO trends by the Director of Quality Assurance.

All SAE and UPRISO's will be reported following MAYS CANCER CENTER, UT HEALTH institutional and FDA guidelines.

UT HEALTH SAE/UPRISO REPORTING REQUIREMENTS For IIS that the PI holds the IND		
Type Event	Report to	Timeframe
All AE, SAE and UPIRSO	Regulatory Affairs and DQA	ASAP
All AE, SAE and UPIRSO	FDA on form 3500A	within 7 calendar days by telephone and 15 calendar days using the Form 3500A
SAE	PI at UT HEALTH	within 24 hours

SAE	UT HEALTH IRB	Annually
UPIRSO - all	PI at UT HEALTH	within 24 hours
UPIRSO - all	FDA	within 7 days
UPIRSO - life threatening	UT HEALTH IRB/UT HEALTH OCR	within 48 hours
UPIRSO - non-life threatening	UT HEALTH IRB/UT HEALTH OCR	within 7 days

AE's and SAE events that occur during clinical trials with or without an Investigational New Drug (IND) application are mandatory reports submitted to FDA via Medwatch FDA F3500A *within 15 days for events that have at least a possible relationship with the drug.*

Additionally, reporting to Agenus for AE's, AESI and SAE's should follow reporting requirements as outlined in the external reporting section.

Assuring Compliance with Protocol and Data Accuracy

As with all studies conducted at MAYS CANCER CENTER, the PI has ultimate responsibility for ensuring protocol compliance, data accuracy/integrity and responding to recommendations that emanate from monitoring activities. Protocol compliance, data accuracy and reporting of events is further ensured by an annual audit conducted by the Data Safety Officer, whose audit report is shared with the PI, the research team, and will be reviewed by the MAYS CANCER CENTER DSMC.

MAYS CANCER CENTER DSMB Membership

The MAYS CANCER CENTER has a primary set of members consisting of UT HEALTH faculty and staff. This Protocol will utilize the Mays CC DSMB for solid tumor studies.

As per NCI guidelines and to eliminate conflict of interest (financial, intellectual, professional, or regulatory in nature), the MAYS CANCER CENTER DSMB specific to this study will not treat patients on this protocol. Usage of the DSMB specific to the histology has been created to ensure that oncology experts in that histology are represented on the DSMB assembled for this protocol, but may be expanded, at the PI's discretion, to include other members which may include:

- experts in the fields of medicine and science that are applicable to the study (if not currently represented on the DSMB),
- statistical experts,
- lay representatives,
- multidisciplinary representation, from relevant specialties including experts such as bioethicists, biostatisticians and basic scientists, and
- others who can offer an unbiased assessment of the study progress.

Additional or alternate membership of in the DSMB is selected by the DSMC chair, in conjunction with the PI of this protocol.

MAYS CANCER CENTER DSMB Charter and Responsibilities

The MAYS CANCER CENTER DSMB will provide information on the membership composition, including qualifications and experience to both the UT HEALTH IRB and MAYS CANCER CENTER PRC for review. The MAYS CANCER CENTER DSMB for this study will act as an independent advisory board to the PI and will report its findings and recommendations to the PI, the UT HEALTH IRB and the MAYS CANCER CENTER DSMB. MAYS CANCER CENTER DSMB reports will utilize the Investigator Initiated Study Quarterly DSMB Report Form and meetings will occur on a monthly basis.

Once the protocol is activated, if not already established elsewhere in the protocol the MAYS CANCER CENTER DSMB will establish and provide:

- procedures for maintaining confidentiality;
- statistical procedures including monitoring guidelines, which will be used to monitor the identified primary, secondary, and safety outcome variables;
- consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study;
- plans for changing frequency of interim analysis as well as procedures for recommending protocol changes;
- recommendation of dose escalation, MTD recommendation of early termination based on efficacy results, or expanding the cohort;
- recommendation of termination due to unfavorable benefit-to-risk or inability to answer study questions;
- recommendation of continuation of ongoing studies;
- recommend modification of sample sizes based on ongoing assessment of event rates; and review of final results and publications.

EXTERNAL REPORTING REQUIREMENTS

The Investigator will report all adverse reactions to the FDA according to 21 Code of Federal Regulations (CFR) 312.32 and according to applicable regulatory authorities and institutional ethics committees.

For SAE's the investigator must notify Agenus immediately (i.e., ≤24 hours after becoming aware) via e-mail and later send the Medwatch FDA F3500A used for submission to FDA by fax, or e-mail.

By electronic media (Adverse.Events@Agenusbio.com)
or
Paper (Fax: +1-781-674-4261).

For the following SAEs the reporting period to Agenus or designee is ≤ 8 hours after becoming aware of the event:

- drug- death related to AGEN2034 or AGEN1884 after less than 30 days of dosing
- AGEN2034 or AGEN1884-related life-threatening event.

Reporting procedures and timelines are the same for any new information on a previously reported SAE (= follow-up).

Pregnancy and in Utero Drug Exposure
Only pregnancies considered by the investigator as related to study treatment (e.g., resulting from a drug interaction with a contraceptive medication) are considered as AEs.

However, all pregnancies with an estimated conception date from first administration to 30-days after the participant's last investigational treatment/intervention must be recorded.

The same rule applies to pregnancies in female subjects and in female partners of male subjects. The investigator must notify Agenus or designee in an expedited manner of any pregnancy using the same process as described for SAE reporting.

Investigator must actively follow up, document, and report the outcome of these pregnancies, even if subjects are withdrawn from the trial. A separate consent will be obtained for follow-up of these subjects.

The investigator must notify Agenus or designee of these outcomes and in case of abnormal outcome, the SAE report form if the subject sustains an event and the parent-child/fetus AE report form when the child/fetus sustains an event).

Any abnormal outcome must be reported in an expedited manner, using the same process as described for SAE reporting, whereas normal outcomes must be reported within 45 days from delivery.

In the event of a pregnancy in a subject occurring during the course of the trial, the subject must be discontinued from trial medication immediately. The Agenus or designee must be notified without delay and the subject must be followed as described above.

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