A phase II trial to evaluate pemetrexed clinical responses in relation to tumor MTAP gene status in patients with previously treated metastatic urothelial carcinoma

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1. Project Overview/Summary:

According to the Cancer Genome Atlas (TCGA), about 25% (21-46%) of bladder tumors harbor homozygous deletion of the MTAP gene (methylthioadenosine phosphorylase). MTAP is an essential enzyme for the salvage pathway of nucleotide synthesis, which is required for formation of DNA/RNA, cell proliferation and survival in the absence of de novo nucleotide synthesis pathway. Pemetrexed has been very well known to block the de novo nucleotide synthesis pathways. Therefore, pemetrexed may be particularly effective for MTAP-deficient malignancies because they lack salvage folate synthesis.

Our preliminary data indicate that pemetrexed, at a concentration as low as 0.1 μM, caused marked apoptosis in 5 MTAP-deficient human bladder cancer cell lines but did not cause significant apoptosis in 5 MTAP wild-type human bladder cell lines or normal human urothelium even at a concentration 200-fold higher (20 μM). On recent retrospective review, 4 patients with MTAP-deficient bladder cancer responded to pemetrexed therapy whereas 8 patients with MTAP wildtype bladder cancer did not responds to pemetrexed. Our animal model as indicates that MTAP gene knockdown tumors grew significantly slower than their wildtype counterparts in mice with pemetrexed treatment. These data strongly support the ***hypothesis*** that MTAP-deficiency in urothelial carcinoma is a determinant of pemetrexed efficacy.

To test this hypothesis, we propose to conduct a Phase 2 clinical trial to evaluate the therapeutic effect of pemetrexed on patients with MTAP-deficient metastatic urothelial carcinoma of the bladder.

These studies are **significant** because they will help establish MTAP deficiency as the first predictive biomarker that can be used to personalize pemetrexed therapy and hopefully prolong the survival of patients with metastatic urothelial carcinoma of the bladder.

2. Introduction

Fifteen thousand of the 75,000 patients with urothelial carcinoma of the bladder will die annually in the US mostly due to metastatic disease (1). The last agent able to lengthen the survival of patients with metastatic urothelial carcinoma was approved more than twenty years ago (2). According to TCGA, approximately 25% (21-46%) of urothelial carcinoma of the bladder harbor homozygous deletion of the MTAP (methylthioadenosine phosphorylase) gene (3-5). Our immunohistochemistry (IHC) study of the MTAP protein using human bladder tumor tissue microarray indicated that 25% (14/56) human bladder tumors were deficient of MTAP protein expression (**Figure 1**). This correlates well with the MTAP gene loss rate according to TCGA.

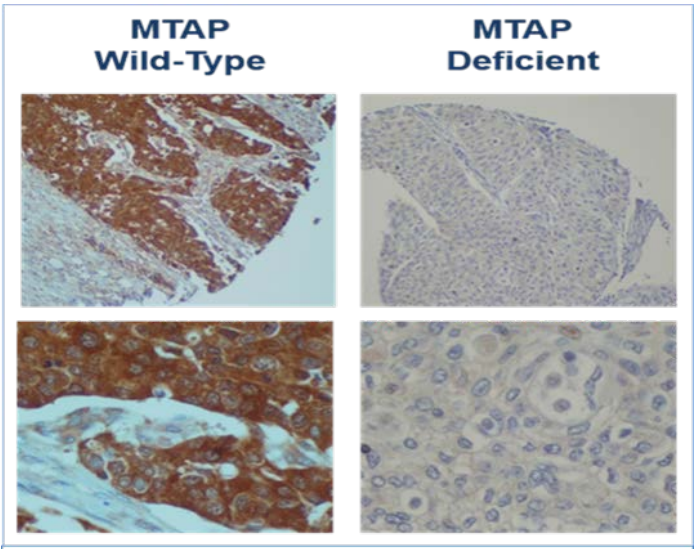


Figure 1. Representative sections of bladder tumor microarray with MTAP-positive and MTAP-negative staining.

The MTAP protein is an essential enzyme for the salvage pathway of nucleotide synthesis, which is required for cell proliferation and survival in the absence of the *de novo* nucleotide synthesis that requires folate synthesis controlled by key enzymes including dihydrofolate reductase (DHFR), thymidylate synthase (TS), and glycinamide ribonucleotide formyltransferase (GARFT) (6). Pemetrexed has been very well known to block folate synthesis and as a result, inhibit de novo nucleotide synthesis. Therefore, MTAP-deficient tumors (which lack salvage nucleotide synthesis) should be particularly vulnerable to pemetrexed (**Figure 2**).

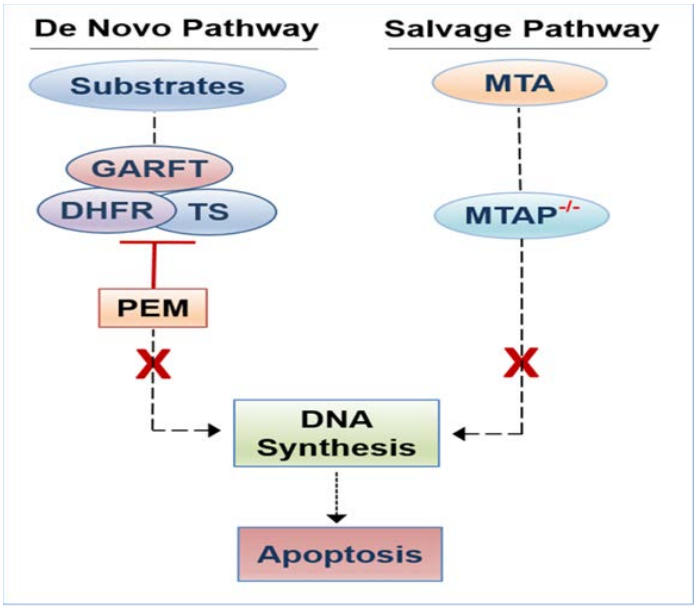


Figure 2. MTAP-deficient bladder cancer may be particularly sensitive to pemetrexed

Pemetrexed has been has been previously tested in a Phase 2 clinical trial in patients with metastatic bladder cancer. As a second-line therapy, pemetrexed showed modest activity in patients with metastatic bladder cancer with a response rate of 28% (13 of a total of 47 patients) (7). It is not known whether or not these pemetrexed-responsive bladder cancers harbor MTAP deletion. However, a response rate of 28% to pemetrexed coincides well with the overall MTAP homozygous deletion rate (25%) in human bladder tumors according to TCGA data and our tumor microarray data (**Figure 1**). These data strongly suggest that bladder cancer with MTAP-deficiency is particularly sensitive to pemetrexed.

Our preliminary data indicate that pemetrexed, at a concentration as low as 0.1 μM, caused marked apoptosis as measured by flow cytometry in 5 MTAP-deficient human bladder cell lines but not in 5 MTAP wild-type human bladder tumor cell lines or normal human urothelium, even at a concentration 200-fold higher (**Figure 3**).

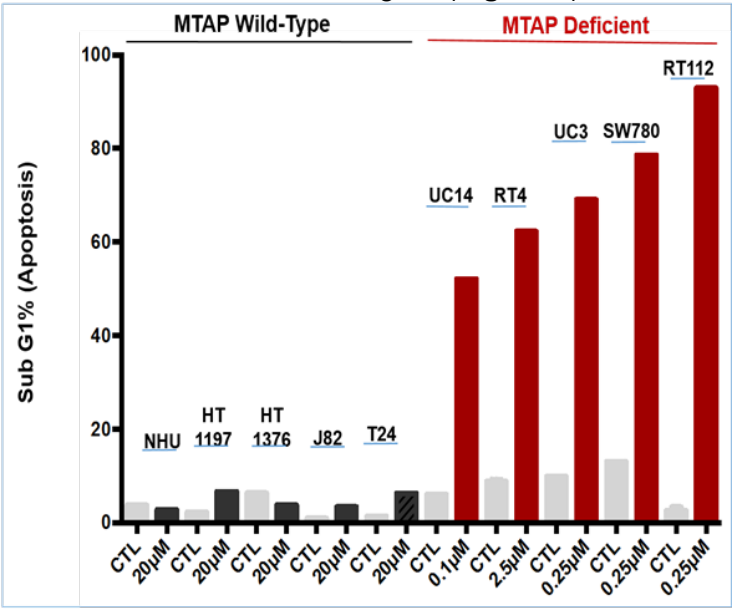


Figure 3. Apoptosis rate in MTAP wild-type vs. MTAP-deficient bladder tumor cell lines in response to pemetrexed as measured by sub-G1 population with flow cytometry; CTL = Control.

In order to assess whether patient clinical responses to pemetrexed correlate with tumor MTAP gene status, we performed retrospective IHC analysis of tumors from 12 patients with metastatic urothelial carcinoma treated with pemetrexed as a second-line therapy. Of these 12 patients, 4 patient had clinical response to pemetrexed and tumor was negative for MTAP IHC staining (**Figure 4A**, representative of one patient). The other 8 patients did not have clinical response to pemetrexed treatment and their tumors were positive for MTAP staining (**Figure 4B,** only one patient's CT scan is shown). Therefore, it appears that clinical responses of metastatic bladder cancer patients treated with pemetrexed correlate with tumor MTAP gene status.

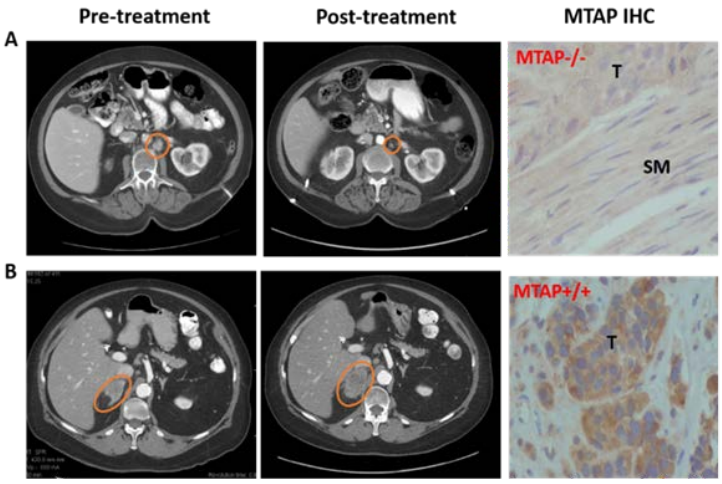


Figure 4. Patient clinical responses to pemetrexed correlate with MTAP deficiency status. Orange circles: Tumor locations; SM-Smooth muscle; T-Tumor.

Collectively, these preliminary data strongly support the ***hypothesis*** that bladder tumors with MTAP gene deficiency are more sensitive to pemetrexed than those with wild-type MTAP gene.

Therefore, we propose this trial to evaluate pemetrexed response rate in patients with MTAP-deficient metastatic bladder cancer. If our hypothesis is validated, data from this trial will serve as a foundation to further test tumor MTAP gene deficiency as the first predictive biomarker to personalize a potentially effective therapy for patients with metastatic urothelial bladder cancer.

3. Primary and Secondary Objectives

The **Primary Objective** of this trial is to determine the objective response rates (ORR) to pemetrexed in patients with MTAP-deficient metastatic bladder cancer.

The **Secondary Objectives** of this trial include:

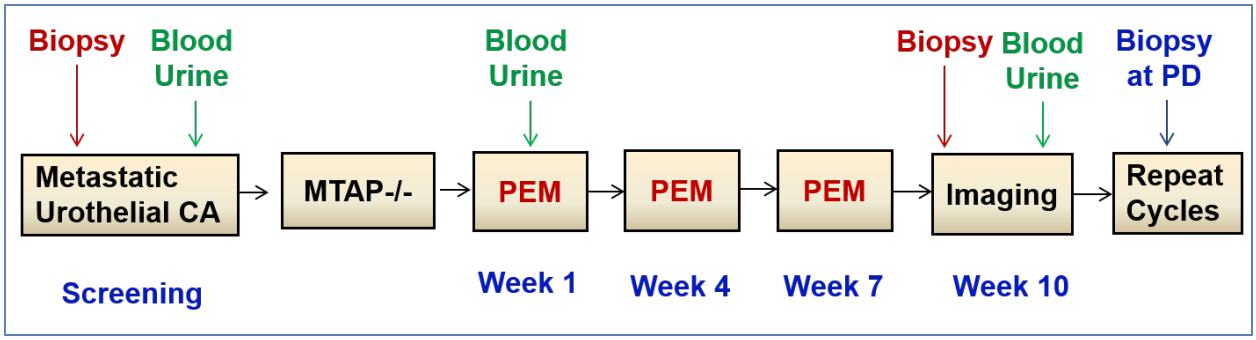
1. To determine the progression-free survival (PFS) for patients with MTAP-deficient metastatic bladder cancer treated with pemetrexed.
2. To determine the overall survival (OS) for patients with MTAP-deficient metastatic bladder cancer treated with pemetrexed.
3. Evaluate the toxicity of pemetrexed therapy for patients with MTAP-deficient metastatic bladder cancer.
4. Collect blood, urine, and tissue for future translational studies.

4. Research Design

4.1.Patients who have metastatic bladder cancer will be subjected to tumor biopsy from the original tumor or metastases to obtain tissue for testing of MTAP status by IHC staining, which will be carried out by Co-PIs Charles Guo and Bogdan Czerniak of the Pathology Department. Of note, we have obtained CLIA certification for MTAP IHC staining. Additional fresh and formalin-fixed tissues will be banked for future translational studies.

4.2. Patients with MTAP-/- tumor will be selected for pemetrexed treatment. A total of 25 patients will be enrolled for this trial.

Trial Schema



4.3.Treatment

Pemetrexed 500 mg/m2 will be administered as an intravenous infusion over 10 minutes on Day 1 of each 21-day cycle.

Folic acid: To reduce toxicity, patients treated with Pemetrexed must be instructed to take a low-dose oral folic acid preparation or multivitamin with folic acid on a daily basis. Dosing should continue during the full course of therapy and for 3 weeks after the last dose of Pemetrexed.

Vitamin B12 (1000 µg) will be administered as an intramuscular injection at the first dose of Pemetrexed and repeated approximately every 9 weeks until 3 weeks after the last dose of study therapy.

Dexamethasone (10 mg) will be administered as an intravenous infusion on Day 1 of each 21-Day cycle.

Dexamethasone (4 mg or equivalent dose of other steroids) twice daily should be taken orally for two days after each dose of Pemetrexed, for rash prophylaxis unless medically contraindicated.

- **Ibuprofen** (400 mg qid) for pain/inflammation control can be administered with Pemetrexed in patients with normal renal function (creatinine clearance > 80 mL/min), caution should be used when administering ibuprofen concurrently with Pemetrexed to patients with mild to moderate renal insufficiency (CrCl from 45 to 79 mL/min). Patients with mild to moderate renal insufficiency should avoid taking NSAIDs with short elimination half-lives for a period of 2 days before, the day of, and 2 days following administration of Pemetrexed. In the absence of data regarding potential interaction between Pemetrexed and NSAIDs with

longer half-lives, all patients taking these NSAIDs should interrupt dosing for at least 5 days before, the day of, and 2 days following Pemetrexed administration. If concomitant administration of an NSAID is necessary, patients should be monitored closely for toxicity, especially myelosuppression, renal, and gastrointestinal toxicity.

- **Leucovorin**: Because folic acid and vitamin B12 supplementation has significantly reduced the number of episodes of Grade 4 hematologic and Grade 3/4 nonhematologic toxicities associated with Pemetrexed therapy, a need for leucovorin as rescue agents is not anticipated. However, this section provides information should rescue be necessary. In clinical trials, leucovorin was permitted for CTC grade 4 leukopenia lasting > 3 days, CTC Grade 4 neutropenia lasting > 3 days, and immediately for CTC Grade 4 thrombocytopenia, bleeding associated with Grade 3 thrombocytopenia, or Grade 3 or 4 muscositis. If needed, leucovorin is given intravenously at 100mg/m2 once, followed by 50 mg/m2 every 6 hours for 8 days.

- **Colony Stimulating Factors**: Growth factors including neupogen (filgrastim) or neulasta (pegfilgrastim) may be used for neutropenia treatment and/or prevention per discretion of the treating physicians.

4.4. Laboratory Monitoring and Dose Reduction/Discontinuation Recommendations

Monitoring: Complete blood cell counts, including platelet counts, should be performed on all patients receiving pemetrexed. Patients should be monitored for nadir and recovery, which were tested in the clinical study before each dose. Patients should not begin a new cycle of treatment unless the ANC is ≥1500 cells/mm3, the platelet count is ≥100,000 cells/mm3, and creatinine clearance is ≥45 mL/min. Periodic chemistry tests should be performed to evaluate renal and hepatic function and at any time clinically necessary per treating physicians. For patients with creatinine clearance ≥ 40, reduced dosing of pemetrexed should be discussed with the pharmacists or PI or Co-PI. In general, pemetrexed dose can be first reduced from 500mg/m^2 to 400mg/m^2 or less as needed to manage toxicity.

Dose Reduction: Dose adjustments at the start of a subsequent cycle should be based on nadir hematologic counts or maximum nonhematologic toxicity from the preceding cycle of therapy. Treatment may be delayed to allow sufficient time for recovery. Upon recovery, patients should be retreated using the guidelines in **Tables 1-2**.

Table 1: Dose Reduction for Pemetrexed– Hematologic Toxicities	
Nadir ANC <500/mm3 and nadir platelets ≥50,000/mm3	75% of previous dose
Nadir platelets <50,000/mm3 without bleeding regardless of nadir ANC	75% of previous dose
Nadir platelets <50,000/mm3 with bleeding regardless of nadir ANC	50% of previous dose

Table 2: Dose Reduction for Pemetrexed– Nonhematologic Toxicities	
Any Grade 3 or 4 toxicities except mucositis	75% of previous dose
Any diarrhea requiring hospitalization (irrespective of Grade) or Grade 3 or 4 diarrhea	75% of previous dose
Grade 3 or 4 mucositis	50% of previous dose

Discontinuation Recommendation: Pemetrexed therapy should be discontinued if a patient experiences any hematologic or nonhematologic Grade 3 or 4 toxicity after 2 dose reductions.

In addition, pemetrexed will be discontinued under the following conditions:

- Disease progression;
- Intercurrent illness that prevents further administration of treatment;
- Patient decides to withdraw from the study;
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigators.

Renally Impaired Patients: Pemetrexed should not be administered to patients whose creatinine clearance is <40 mL/min using the standard Cockcroft and Gault formula (below) or GFR measured by Tc99m-DTPA serum clearance method:

Males: [(140 – Age in years) x Actual Body Weight (kg)] / [Serum Creatinine (mg/dL)×72]

Females: Estimated creatinine clearance for males × 0.85

When in doubt (e.g. GFR at borderline levels of 40), we will confirm the above calculation using the body surface area (BSA)-adjusted CKD-EPI formula, which has been recently shown to more accurately estimate GFR, especially in patients with chronic kidney disease (8).
GFR = 141 × min(Scr/κ, 1)α × max(Scr/κ, 1)-1.209 × 0.993Age × 1.018 [if female] _ 1.159 [if black], where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κor 1, and max indicates the maximum of Scr/κ or 1.

Caution should be exercised when administering pemetrexed concurrently with NSAIDs to patients whose creatinine clearance is <80 mL/min as stated in Section 4.3.

For patients with creatinine clearance ≥ 40 but < 45 reduced dosing of pemetrexed should be discussed with the pharmacists or PI or Co-PI. In general, pemetrexed dose can be first reduced from 500mg/m^2 to 400mg/m^2 or less as needed to manage toxicity.

4.5.Other treatment

Patients who have complete response after being treated with pemetrexed may undergo consolidation surgery to eradicate possible residual cancer, as a standard practice at the M. D. Anderson Cancer Center (MDACC). Tissues from consolidation surgery will be collected and banked for immunologic and molecular studies.

Subsequent pemetrexed treatment may be carried out at the MDACC local Regional Cancer Centers or local physician office. However, initial screening, workup, the first dose of pemetrexed, restaging, and biological sample collection must be done at the MDACC main campus. Patients with local physicians who agree to manage standard infusions must agree to perform required tests, send all related medical records to MDACC, and allow the MDACC investigator to direct dose adjustments.

4.6.Sample collection

Peripheral blood and urine will be collected at baseline, the first day of pemetrexed therapy, and every 3 cycles of pemetrexed or restaging thereafter. Tumor biopsy will be obtained at baseline, after 3 cycles of pemetrexed treatmentand at disease progression for evaluation of the effects of pemetrexed on the tumor microenvironment and possible resistance mechanisms to pemetrexed and to guide further therapy as indicated in the trial schema above. Past results on MTAP status of tumor tissue can be used to determine eligibility for inclusion. Archival tissue from prior tumor biopsy can be used for MTAP testing. If clinically safe for patients, we will obtain at least 3 large cores of fresh frozen tumor tissues in addition to FFPE samples for analyses.
Blood, urine, and tissues will be collected on a separate IRB-approved laboratory protocol at the Department of Genitourinary Medical Oncology at MDACC for immunologic and molecular studies.

4.7.Follow up

Patients will be followed for 2 years after last dose or until death, whichever occurs first.

5. Inclusion Criteria

1. Patients must have histological confirmation of metastatic urothelial carcinoma. Patients must have sufficient tumor tissues for future MTAP testing and research. Histological variants such as glandular, squamous, sarcomatoid, micropapillary, plasmacytoid, and small cell changes will not be allowed for this trial unless these tumors are MTAP-deficient.
2. All patients must have measurable disease and tumors of sufficient sizes for biopsy. In general, liver and lung lesions should be at least 1.0 cm, and patients with lymph node-only disease should have lesions of $\geq$ 1.5 cm in shortest dimension. Patients with disease confined to bone may be eligible if a measurable lytic defect is present. The study PI is the final arbiter in questions related to measurability. Patients with a three-

dimensional mass or pelvic sidewall fixation on bladder examination under anesthesia are considered to have measurable disease.

3. Patients who have received any non-anti-folate containing neoadjuvant or systemic chemotherapy are eligible. Any prior intravesical therapy, or immunotherapy is allowed.
4. Patients must have an ECOG performance status ≤ 2.
5. Adequate liver function as defined by AST or ALT ≤ 3 x ULN, or ≤ 5 x ULN if documented liver metastases are present.
6. Total bilirubin ≤ 1.5 x ULN, except subjects with Gilbert's syndrome or liver metastases, who must have a baseline total bilirubin ≤ 3.0 mg/dL.
7. Adequate bone marrow reserves as define by an ANC ≥ 1500, and platelets ≥ 100,000.
8. Adequate renal function as defined by a normal serum creatinine, or a creatinine clearance ≥ 40 ml/min [either measured using a 24 hour urine, calculated using Cockroft-Gault, or estimated using the MDRD method from the National Kidney Disease Education Program (NKDEP) (the method reported by MDACC laboratories)]

   - Cockroft-Gault formula: CL_{cr} = [(140-age) • wt(kg)]/[72 •Creat (mg/dL)] (Multiply by 0.85 for females)

9. Females of childbearing potential who are sexually active with a non-sterilized male partner and non-sterilized males must use a highly effective method of contraception for 28 days prior to the first dose of investigational product, and must agree to continue using such precautions for 180 days after the final dose of investigational product; cessation of contraception after this point should be discussed with a responsible physician. They must also refrain from egg cell donation for 180 days after the final dose of investigational product;
   a. Females of childbearing potential are defined as those who are not surgically sterile (ie, bilateral tubal ligation, bilateral oophorectomy, or complete hysterectomy) or postmenopausal (defined as 12 months with no menses without an alternative medical cause);
   b. A highly effective method of contraception is defined as one that results in a low failure rate (ie, less than 1% per year) when used consistently and correctly. The acceptable methods of contraception are: Barrier Method (e.g. male condom with spermicide, copper T intrauterine device, or levonorgesterl-releasing intrauterine system - Mirena®) or Hormonal Methods (e.g. implants, hormone shot or injection, combined pill, minipill, or patch).

   Non-sterilized males who are sexually active with a female partner of childbearing potential must use a highly effective method of contraception (**Table 3**) from Days 1 through 180 post last dose. In addition, they must refrain from sperm donation for 180 days after the final dose of investigational product.
10. The ability to interrupt NSAIDS 2 days before (5 days for long-acting NSAIDs), the day of, and 2 days following administration of Pemetrexed.
11. The ability to take folic acid, Vitamin B12, and dexamethasone according to protocol.

Table 3 Highly Effective Methods of Contraception

Barrier Methods	Hormonal Methods
• Male condom with spermicide • Copper T intrauterine device • Levonorgesterel-releasing intrauterine system (eg, Mirena®) [a]	• Implants • Hormone shot or injection • Combined pill • Minipill • Patch

[a] This is also considered a hormonal method.

6. Exclusion Criteria

1. Primary central nervous system (CNS) malignancies or CNS metastases, including leptomeningeal metastases, are not allowed. Subjects with previously treated brain metastases will be allowed if the brain metastases have been stable for at least 2 months following prior treatment (radiotherapy or surgery).
2. Patients who received previous anti-folate-containing chemotherapy.
3. Any other malignancy from which the patient has been disease-free for less than 2 years, except for non-melanoma skin cancer, controlled localized prostate cancer, in situ carcinoma of any site.
4. Women who are pregnant or breastfeeding.
5. Presence of third space fluid which cannot be controlled by drainage. For patients who develop or have baseline clinically significant pleural or peritoneal effusions (on the basis of symptoms or clinical examination) before or during initiation of Pemetrexed therapy, consideration should be given to draining the effusion prior to dosing. However, if, in the investigator's opinion, the effusion represents progression of disease, the patient should be discontinued from study therapy.

7. Schedule of Assessments

Parameter	Day -7 to -14	Pemetrexed C1D1	Subsequent cycles (+/- 7 days)	End of therapy (+/- 3 weeks)
Informed Consent	X[a]			
History and Physical	X[a]	X[j]	X[k]	X[k]
Height and Weight	X[a]	X[j]	X	X
Blood Pressure	X[a]	X[j]	X	X
Performance Status	X[a]	X[j]	X	X

Start Vitamin B12 and folic acid	X[b]		X[b]	
CBC, Plt, differential	X[c]	X[j]	X	X
Lytes, BUN, Cr, GFR	X[c]	X[j]	X	X
AST or ALT, alk phos, Total bilirubin	X[c]	X[j]	X	X
bHCG[c]	X[d]			
CT or MRI of chest/abd/pelvis	X[a,e]		X[e]	X[m]
Bone scan	X[a,f]		X[f]	X[m]
PET imaging	X[g]		X[g]	X[m]
Research Blood/Urine collection	X[h]	X	X	
Tissue collection	X[i]			X[i]
Survival assessment[l]				X

a. Must be done within 4 weeks prior to registration; disease focused history with assessment of major comorbidities
b. Vitamin B12 (1000 μg) IM injection x1 should be started at C1 pemetrexed treatment and then repeat every 9 weeks until 3 weeks after finishing the last dose of pemetrexed. Folic acid (400 μg) po daily must be taken at the first dose of Pemetrexed; and dosing should continue during the full course of therapy and for 3 weeks after the last dose of pemetrexed.
c. Blood work must be done within 14 days of treatment.
d. Serum bHCG will be obtained only in females of childbearing potential to be used as confirmation of a lack of pregnancy. However, positive bHCG does not necessarily indicate pregnancy as bladder cancer often produces bHCG. Women of reproductive potential who have elevated bHCG that may be due to their bladder cancer will have repeat bHCG within 2 weeks. If bHCG increases rapidly (usually >1000 mIU/ml and doubles or triples within a week in the case of pregnancy but <1000mIU/ml in bladder cancer), these patients will be refer to Gynecology for further evaluation (e.g. with uterine ultrasound) to ensure that they are not pregnant prior to entering study.
e. CT or MRI of chest/abdomen/pelvis is to be done at baseline within 4 weeks prior to registration and then after very 3-cycles of pemetrexed therapy or whenever clinically needed per treating physician.
f. A bone scan is not routinely required at baseline, but should be done in the presence of bone symptoms or an alkaline phosphatase > 1.5x ULN
g. PET imaging should be done whenever possible to evaluate for metabolic changes in tumor, as allowed by the insurance company.

h. Blood (up to 50ml) and urine samples (≥ 50ml) will be collected at baseline, the first day of pemetrexed therapy, and every 3 cycles of pemetrexed/restaging thereafter.
i. Baseline tissue will be collected by core needle/excision biopsy by Interventional Radiology or transurethral bladder tumor resection if the primary tumor is in place in the bladderfor MTPA IHC staining and research. Tumor tissue biopsy will be obtained at disease progression for evaluation of changes in tumor biology to guide future therapies and study resistance mechanisms.
j. Course 1 day 1 labs do not need to be repeated if completed within 7 days of dosing.
k. Height will be checked every 3 cycles. Height is not required at the end of study.
l. Survival will be assessed every 3 months by clinic visit, chart review, or brief phone call
m. Diagnostic scans should be repeated for confirmation of disease status per treating physician discretion at end of study if greater than 4 weeks since the last assessment.

8. Response Assessment

The primary objective of this study is to determine the ORR of patients with MTAP-deficient vs. MTAP wild-type bladder cancer treated with Pemetrexed. Evaluation of response will follow the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Guidelines as detailed in **Appendix 1**. All tumor measurements must be recorded in centimeters.

9. Statistical Considerations

9.1 Preliminaries

This is a single arm, open-label phase II trial to determine the ORR of pemetrexed in patients with metastatic, MTAP-deficient (MTAP-/-) bladder cancer.

9.2 Endpoints (Measures)

9.2.1 Primary Endpoint:

Overall Response Rate (ORR)

ORR is defined as the number of subjects with a best response of CR or PR at any protocol evaluation by RECIST 1.1 criteria divided by the total number of subjects receiving their first dose of trial therapy.

9.2.2 Secondary Endpoints:

Progression-Free Survival (PFS)

PFS is defined as the time from trial entry to the first documented tumor progression as determined by the investigator using the RECIST 1.1 criteria or death from any cause, whichever occurs first. Patients who are alive and free of known progression at the time of analysis will be censored on the date of last tumor assessment.

Overall Survival (OS)

OS is defined as the time from trial entry to death from any cause. Patients who are alive at the time of analysis will be censored on the date of contact.

Adverse Events (AE)
AEs will be recorded according to section 10 below.

9.3 Sample Size Considerations

A maximum of 25 MTAP-/- patients will be enrolled from MDACC, at an estimated accrual rate of 1-2 patients per month. The sample size of 25 patients allows that if the trial continues to full size and has a response rate of 40% for PEM, the Bayesian posterior 95% credible interval will be (22%, 58%) using a prior of beta (0.56, 1.44), as described in section 9.4. Interim monitoring for futility and efficacy will be performed once 10 patients are evaluable, with continuing enrollment during analyses. After the first analysis, the trial will be monitored every 2 months with continuous entry. If the true response rate is 40%, the trial will stop early 14% of the time. If it's very bad, like 20%, the trial will stop 79% of the time, and if it's as high as 60% it will stop less than 1% of the time. Additional operating characteristics for various scenarios are provided in Table 9.4.2 below.

9.4 Interim Monitoring

9.4.1 Futility Monitoring

Ongoing monitoring will be implemented based on the method of Thall (1995)(9) every other month once the first 10 patients have become evaluable. Calculations were performed in Multc Lean 2.1. Patients will be counted as evaluable for monitoring if they have had their 10 week imaging, have dropped out of the study prior to 10 weeks, have progressed prior to 10 weeks, or if 12 weeks have passed since trial entry without any imaging, unless there is documented treatment delay and image is still pending. Once 10 MTAP wt patients are evaluable, the following monitoring calculations will be performed on all patients who are evaluable.. Accrual will not be held during the boundaries check. Denote the probability of ORR by θ_R. We assume θ_R ~ beta (0.8, 1.2). Our stopping rule is given by the following probability statement: $Pr(\theta_R < 0.40 \mid data) > 0.95$. That is, we will stop entering patients if, at any time during the study, we determine that there is more than an 95% chance that the ORR is less than 40%, a constant rate that is minimum interesting response rate for these patients if we are correct that this genotype is more likely to respond. The stopping boundaries for this futility rule are to terminate enrollment if the number of overall responses compared to the number of patients who are evaluable meets the stopping boundaries of Table 4 below. Monitoring will be carried out once there are 10 patients who have been treated and evaluated. Patients who leave the study after receiving treatment but before the 10 week evaluation will be counted as non-responders and therefore evaluable for response. Patients who enroll but never receive treatment will be replaced.

Table 4. Stopping Criteria for Insufficient Responses

If there are this many (or more) evaluable for ORR:	**10**	**12**	**16**	**19**	**22**	**25**
Stop if there are this many patients (or fewer) with an objective response:	**1**	**2**	**3**	**4**	**5**	**6***

*Always stop with 25 patients, but if 6 or fewer patients respond, then this combination is not interesting for further investigation in these patients.

Table 5. The Operating Characteristics under Potential Population Response Rates

True Overall Response Rate	Probability of Stopping Early	Probability of Continuing after 10 Patients	Median (25^{th} %ile, 75^{th} %ile)
0.10	0.99	0.26	10 (10, 12)
0.20	0.79	0.62	12 (10, 22)
0.30	0.42	0.85	25 (12, 25)
0.40	0.14	0.95	25 (25, 25)
0.50	0.03	0.99	25 (25, 25)
0.60	0.004	0.998	25 (25, 25)

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9.4.3 Safety Monitoring

Pemetrexed is an approved and safe treatment with a known toxicity profile. No early stopping rules for safety will be performed.

9.5 Statistical Analyses Plan

Descriptive statistical analyses will be performed to summarize the ORR and individual adverse event rates with 95% credible intervals, with priors of beta(0.8, 01.2) and (1, 1), respectively. Additionally, ORR will be similarly reported for a treated subgroup of patients receiving at least 75% of planned therapy for the first 3 cycles. PFS and OS will be estimated and plotted with the methods of Kaplan and Meier (10). As a planned secondary analysis, the PFS and OS estimates at 2 years will be reported with their respective standard errors.

10. Adverse Events

Adverse events will be graded according to the CTCAE 4.0 with the level of attribution to the study drug assigned by the attending physician.

10.1 Serious Adverse Event Reporting (SAE)

An adverse event or suspected adverse reaction is considered “serious” if it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32)

- Important medical events as defined above may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Investigator.
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in “The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Serious Unanticipated Adverse Events for Drugs and Devices”.
- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, and Institutional Review Board policy.

10.2 Reporting of Adverse Events

Adverse Events will be documented according to the Recommended Adverse Event Recording Guidelines for Phase II protocol (see table below).

Recommended Adverse Event Recording Guidelines						
Attribution	Grade 1	Grade 2	Grade 3		Grade 4	Grade 5
Unrelated	Phase I	Phase I	Phase I Phase II		Phase I Phase II Phase III	Phase I Phase II Phase III
Unlikely	Phase I	Phase I	Phase I Phase II		Phase I Phase II Phase III	Phase I Phase II Phase III
Possible	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III		Phase I Phase II Phase III	Phase I Phase II Phase III
Probable	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III		Phase I Phase II Phase III	Phase I Phase II Phase III
Definitive	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III		Phase I Phase II Phase III	Phase I Phase II Phase III

10.3 Reporting Requirements to Supporting Company:

This trial will use the NCI-CTCAE v4.0 criteria for assessment of toxicity and SAE reporting with regard to toxicity grade.

10.3.1 Adverse Events

Investigators should refer to Eli Lilly's prescribing information for the expected side effects of pemetrexed. As with any agent, there is always the potential for unexpected AEs, including hypersensitivity reactions.

Therapeutic monitoring should be performed following pemetrexed administration in a manner consistent with the local clinical standard of care. In general, subjects should be closely monitored for side effects of all concomitant medications.

The Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

10.3.2 Reporting of Serious Adverse Events

IND-exempt studies: All serious adverse events (regardless of relationship or expectedness) will be reported and documented on MD Anderson SAE form.

Each serious adverse event must be followed up until resolution or stabilization, by submission of updated reports to the designated person. An isolated laboratory abnormality that is assigned grade 4, according to CTC definition, is not reportable as an SAE; unless the investigator assesses that the event meets standard ICH criteria for an SAE. CTC grade 4 baseline laboratory abnormalities that are part of the disease profile should not be reported as an SAE, specifically when they are allowed or not excluded by the protocol inclusion/exclusion criteria.

10.4 Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the study team (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

11. Data Management and Confidentiality Plan

All data will be entered to the Department of Genitourinary Medical Oncology database, which is a password protected database with an audit trail. The database meets regulatory compliance. Data can be collated with a unique identification in order to de-link information. The minimum required fields will be entered to the institutional required data collection systems. Informed consent and registration data entry will occur prior to protocol specific events. All eligibility criteria must be satisfied.

12. Publication Strategy

Preliminary data from this clinical trial will be presented at national meetings such as ASCO or GU ASCO annual meetings. If the final data confirm our hypothesis, we believe that data from this trial can be published in a high-impact journal such as New England Journal of Medicine.

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Appendix 1. Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Guidelines

- Target Lesions:

All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (those with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter for all target lesions will be calculated and reported as the baseline sum longest diameter. The baseline sum of longest diameters will be used as the reference by which the objective tumor response is characterized.

- Non-target Lesions:

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

- Evaluation of Target Lesions:

Complete Response: The disappearance of all target lesions.

Partial Response: At least a 30% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum longest diameter.

Progressive Disease: At least a 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since the treatment started or the appearance of one or more new lesions.

Stable Disease: Insufficient shrinkage to qualify for partial response, or insufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter since the treatment started.

- Evaluation of Non-target Lesions:

Complete Response: The disappearance of all non-target lesions.

Incomplete Response/Stable Disease: The persistence of one or more non-target lesion(s)

Progressive Disease: The appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

- Evaluation of Best Overall Response:

The best overall response is the best response recorded from the start of treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started) as shown in the following table.

Target Organ Lesions	Non-Target Organ Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Patients with symptoms attributable to progressive cancer (ie: increasing bone pain) will be counted as progression, regardless of the marker.

- Time -to- Event Assessment of Response
- Time to Response: From the start of study drug to the first observation of a response (the first of two confirmatory measurements).
- Duration of Response: From the first observation of a response (the first of the two confirmatory statements) to the first observation of progressive disease, or to death due to any cause, or early discontinuation of treatment due to progressive disease.
- Time to Progression: From the start of study drug to the first evidence of progression.
- Survival: Survival will be calculated from the start of the study drug to death due to any cause.
- Disease free survival will be calculated.