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A Window of Opportunity Trial: Avelumab in Non-metastatic Muscle Invasive Bladder Cancer (BL-AIR: Bladder cancer-Avelumab for Invasive Resectable disease)

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ABBREVIATIONS AND DEFINITIONS OF TERMS

1. A2AR = adenosine A2a receptor
2. ADCC = antibody dependent cellular cytotoxicity
3. Adeno = adenocarcinoma
4. AEs = adverse events
5. AESIs = adverse events of special interest
6. BC = bladder cancer
7. BCM = Baylor College of Medicine
8. BSC = best supportive care
9. BTLA4 = B and T lymphocyte attenuator
10. C = Cycle
11. CBP = child bearing potential
12. CCL = C-C motif chemokine ligand
13. CD = cluster of differentiation
14. Chemo = chemotherapy
15. CMV = cisplatin + methotrexate + vinblastine
16. CT =computed tomography
17. CTCAE = common terminology criteria for adverse events
18. CTLA-4 = cytotoxic T-lymphocyte protein 4
19. CXCL = C-X-C motif chemokine ligand
20. D = day
21. DAMP = damage-associated molecular pattern
22. DFS = disease free survival
23. DLDCCC = Dan L Duncan Comprehensive Cancer Center
24. DSS = disease specific survival
25. ELISA = enzyme-linked immunosorbent assay
26. EPO = erythropoietin
27. FDA = federal drug administration
28. FFPE = formalin fixed paraffin embedded
29. FOXP3 = forkhead box P3
30. F/U = follow-up
31. GC = gemcitabine + cisplatin
32. G-CSF = granulocyte macrophage colony stimulating factor
33. GE = gastroesophageal
34. GM-CSF = granulocyte macrophage colony stimulating factor
35. ICI = immune checkpoint inhibitors
36. IDO = indoleamine 2-3 dioxygenase
37. IFN-γ = interferon gamma
38. IHC = immunohistochemistry
39. IRB = institutional review board
40. KIR = killer immunoglobulin receptor
41. LAG3 = lymphocyte activation gene 3
42. ICH = International Conference on Harmonization
43. ICOS = inducible T-cell costimulatory
44. IgG1 = immunoglobulin G1

45. IRB = institutional review board
46. iNOS = inducible nitric oxide synthase
47. IL = interleukin
48. m = months
49. mABs = monoclonal antibodies
50. M-CSFR = macrophage colony stimulating factor receptor
51. MDSCs = myeloid derived suppressor cells
52. MHC = major histocompatibility
53. MIBC = muscle invasive bladder cancer
54. MRI = magnetic resonance imaging
55. MVAC = methotrexate + vinblastine + adriamycin + cisplatin
56. NA = neoadjuvant
57. N/A = not available
58. NAC = neoadjuvant chemotherapy
59. NMIBC = non-muscle invasive bladder cancer
60. NSCLC = non-small cell lung cancer
61. NK = natural killer
62. ORR = overall response rate
63. OS = overall survival
64. PD-1 = programmed death 1
65. PD-L1 = programmed death ligand 1
66. PD-L2 = programmed death ligand 2
67. PGE2 = prostaglandin E2
68. PLND = pelvic lymph node dissection
69. PFS = progression free survival
70. pT0 = pathologic complete response
71. RC = radical cystectomy
72. RCT = randomized controlled trial
73. RECISTv1.1 = response evaluation criteria in solid tumors version 1.1
74. RNA = ribonucleic acid
75. SAE = serious adverse event
76. SC = squamous cell carcinoma
77. SITC = society for immunotherapy in cancer
78. T-bet = t box protein in B cells
79. TGF-β = transforming growth factor β
80. TIM3 = T-cell membrane protein 3
81. TNBC = triple negative breast cancer
82. TNF = tumor necrosis factor
83. TPO = thrombopoetin
84. TTP = time to progression
85. TURBT = transurethral resection of the bladder tumor
86. UCB = urothelial cancer of the bladder
87. UCC = urothelial cell carcinoma
88. ULN = upper limit normal
89. VEGF = vascular endothelial growth factor
90. wks = weeks

1. INTRODUCTION

1.1 Role of Neoadjuvant Chemotherapy in Muscle Invasive Bladder Cancer

Multiple clinical trials have shown improvements in overall survival (OS) and disease free survival (DFS) for patients with muscle invasive bladder cancer (MIBC) administered cisplatin containing neoadjuvant chemotherapy (NAC) when compared to radical cystectomy (RC) alone. (Table 1) (1-11) In these studies improved survival has been associated with lack of residual disease at the time of RC. (2-10) Additionally, multiple meta-analyses have shown significant benefits for cisplatin containing NAC when compared to RC alone. One of these meta-analyses consisted of 3005 patients across 11 trials; it showed 5 year absolute improvements for OS of 5% and DFS of 9%. (12)

Traditionally the NAC of choice was standard dose methotrexate + vinblastine + adriamycin + cisplatin (MVAC), but recently studies have suggested equivalency of gemcitabine + cisplatin (GC) and dose-dense MVAC. (Table 1) The three cisplatin containing NAC regimens recommended by the National Comprehensive Cancer Network include 1) dose-dense MVAC for 3-4 cycles, 2) GC for 4 cycles or 3) cisplatin + methotrexate + vinblastine (CMV) for 3-4 cycles. (13) The regimen we generally use neoadjuvantly for MIBC at Baylor affiliates is GC. These regimens and the data in Table 1 are for those with predominantly urothelial cell carcinoma (UCC), which is the majority of the patients seen in clinical practice and those that will be included in our trial. Perioperative recommendations for other histologies (e.g., squamous cell and adenocarcinoma) are different and are not discussed here since those patients without predominant UCC histology are excluded from participation in our trial.

Table 1: Studies of Cisplatin Containing Neoadjuvant Chemotherapy in Muscle Invasive Bladder Cancer

Study	Study Type	Patient Number	NAC Treatment	pT0 Rate	OS
Grossman HB et al.	Phase III RCT	317	3 cycles standard dose MVAC	a) NAC – 38%, p < 0.001 b) No NAC – 15%	c) NAC – median 77m, p = 0.05 d) No NAC – median 46m
BA06 30894 trial	Phase III RCT	976	3 cycles of CMV	a) NAC – 32.5% b) No NAC – 12.3%	c) NAC – 10 year 36%, HR 0.84, p = 0.037 d) No NAC – 10 year 30%
Pilmack ER et al.	Phase II	40	3 cycles of dose-dense MVAC	38%	N/A
Herchenhorn D et al.	Phase II	22	3 cycles of GC	26.7%	Median 36m

Fairey AS et al.	Retrospective	116	4 cycles of GC (n = 58) or 4 cycles of standard dose MVAC (n = 58)	a) GC – 21% b) MVAC – 10%	a) GC – Median 24.8m b) MVAC – Median 26.4m, p = 0.634
Galsky MD et al.	Retrospective	212	3 cycles GC (n = 146) or 3 cycles MVAC (77% dose-dense) (n = 66)	a) GC – 31%, p = 0.77 b) MVAC – 29%	a) GC – Median 26.8m b) MVAC – Median 35.5m, p = 0.17
Van de Putte EE et al.	Retrospective	80	4 cycles dose-dense MVAC	29%	N/A
Yeshchina O et al.	Retrospective	114	MVAC (n = 77) or GC(n = 37)	a) MVAC – 31%, p = 0.645 b) GC – 25%	a) MVAC – 5 year 47%, p = 0.346 b) GC – 5 year 35%
Yuh BE et al.	Meta-analysis	164	GC	25.6%	N/A

NAC = neoadjuvant chemotherapy, pT0 = pathologic complete response, OS = overall survival, RCT = randomized controlled trial, MVAC = methotrexate + vinblastine + adriamycin + cisplatin, m = months, CMV = cisplatin + methotrexate + vinblastine, N/A = not available, GC = gemcitabine + cisplatin

NAC regimens containing carboplatin instead of cisplatin have not been demonstrated equivalent to cisplatin based NAC. (13) Limited data in the advanced, unresectable setting has suggested substituting carboplatin for cisplatin in combination regimens may actually be inferior. (14) Because of lack of data it is not recommended to use carboplatin in the NAC setting. (13) Investigations are currently ongoing to see if carboplatin based NAC regimens could provide a benefit in patients whom are not candidates for cisplatin; however, carboplatin use in the NAC setting is not currently part of standard of care. For those unable or unwilling to receive cisplatin containing NAC the standard of care is to proceed directly to RC, as there is no alternative systemic regimen accepted in the NA setting.

1.2 Overview of Immune Checkpoint Inhibition and Implications in Bladder Cancer Carcinogenesis

Immune checkpoints regulate the ability of T-cells to act against tissues or cells expressing specific antigens. This has benefit in that inhibitory checkpoints help limit T-cell effector functions against self-tissues. However, some tumors can take advantage of this mechanism and over-express inhibitory proteins in the immune checkpoint pathway (both on tumor cells themselves and in the tumor microenvironment) leading to tumor evasion of the immune system and lack of effector T-cell attack against tumors. (15)

CD8+ T-cells are involved in the cell-mediated component of antigen recognition and CD4+ T-cells have roles in both cell-mediated/humoral immune responses. New anti-cancer drugs targeting inhibitory immune checkpoints are revolutionizing cancer therapy. These drugs do not

target tumors directly, rather they target lymphocyte receptors or their ligands to enhance endogenous anti-tumor immunity. (15) While there are multiple immune checkpoints, the two most well-known pathways include the programmed death 1 (PD-1) pathway (CD279) and the cytotoxic T-lymphocyte protein 4 (CTLA4) pathway (CD152). CTLA4 is a receptor on T-cells that when bound by its activating ligands (CD80 and CD86) inhibits the effector function of T-cells and prevents proper costimulatory signaling via CD28, resulting in decreased effector T-cell function. (15) Ipilimumab is a Federal Drug Administration (FDA) approved drug that is an inhibitor of CTLA4. This drug has demonstrated improved OS when compared to standard chemotherapy in advanced melanoma; however, it has increased rates of high grade adverse events and immune-mediated adverse events when compared to antibodies against PD-1 or PD-L1. (16-17)

Immune checkpoint inhibitors (ICIs) targeting the PD-1 pathway are more widely utilized and more effective than those targeting CTLA4. Additionally, PD-1 pathway inhibition appears to have less side effects than that of CTLA4 inhibition. Activation of PD-1 is much more specific than CTLA4 with regards to inhibiting T-cell effector functions against a particular stimulus. PD-1 is a cell surface receptor located on T-cells, B-cells and natural killer (NK) cells. Thus, PD-1 inhibition can also enhance lytic activity of NK cells against tumors and antibody production by B-cells. The two ligands for this receptor are programmed death ligand 1 (PD-L1 or CD274) and programmed death ligand 2 (PD-L2 or CD273). PD-L1 is the more studied of these ligands. (15)

PD-L1 expression on tumor cells and other cells within the tumor microenvironment may be induced by interferon-γ (IFN-γ) or result from genetic changes within tumor cells themselves. IFN-γ is secreted by effector T-cells targeting the tumor and this is why some have hypothesized that increased PD-L1 staining may be reflective of a stronger antitumor immune response waiting to be unleashed by ICIs. (18-19) Several studies have suggested increased response rates in tumors where the cancer cells and the immune infiltrates express higher amounts of this ligand, and some have even suggested improved survival with increased staining of PD-L1. (20-22) There are currently inhibitory antibody drugs against both PD-1 and PD-L1.

Other immune checkpoints include lymphocyte activation gene 3 (LAG3), T-cell membrane protein 3 (TIM3), adenosine A2a receptor (A2AR), killer immunoglobulin receptor (KIR) and B and T lymphocyte attenuator (BTLA). (15) There are drugs in early clinical development against LAG3 and in certain tumor types this checkpoint has been suggested to be involved in dampening the antitumor response that is elicited by agents blocking the PD-1 pathway. (23-24) LAG3 has also been shown to be overexpressed in some bladder cancers. (25) Thus, because of these aforementioned points we plan to examine LAG3 in our study.

In contrast to traditional chemotherapies, ICIs may have a longer time to response and in rare cases it may appear as if lesions are enlarging due to the immune cells infiltrating the tumor. Thus, some have proposed different response criteria to evaluate their effectiveness. (15) The most exciting thing about these checkpoint inhibitor drugs is the prolonged anti-tumor responses they produce and the potential to transform some cases of metastatic cancer into chronic diseases.

Both the PD-1 pathway and CTLA-4 pathway have been suggested to be involved in the transition from non-muscle invasive bladder cancer (NMIBC) to muscle invasive bladder cancer (MIBC). In MIBC PD-1 expression is 59.5%, versus 22.5% in NMIBC and 6.7% in normal bladder tissue. PD-L1 expression in MIBC is 60.7% versus 4.2% in NMIBC and 0% in normal

bladder. Similarly, CTLA4 was overexpressed in 84.5% of MIBC versus 20% of normal bladder tissue. One of the ligands for CTLA4, CD80, is overexpressed in 92.9% of MIBC versus 46.5% of NMIBC and 6.7% of normal bladder tissue. (26)

1.3 Treatment with Immune Checkpoint Inhibitors in Bladder Cancer

Only one ICI has been evaluated previously in the NA setting for MIBC. This ICI was ipilimumab, which is an inhibitory antibody to CTLA-4. 12 patients were evaluated. Some of these patients were chemotherapy candidates and received adjuvant platinum based therapy. While no long-term survival data were reported, the combination was found to be safe and tolerable. The use of pre- and post-treatment biopsies allowed for analysis of antitumor immune responses to ICIs and correlation with outcome parameters. They found that the infiltrating cells seen in response to ipilimumab treatment consisted predominately of CD4+ and CD8+ T-cells, with very few B cells or NK cells. They also found that greater levels of CD4+ T-cells with high expression of inducible T-cell costimulatory (ICOS) receptor in tumor tissue and systemic circulation correlated with improved outcomes when compared to levels of CD4+ T-cells with lower expression of ICOS. (27)

Recent data suggests that ICIs targeting PD-L1 or PD-1 (e.g., avelumab, atezolizumab, durvalumab, nivolumab and pembrolizumab) may provide good responses and survival outcomes in metastatic BC after progression on cisplatin-based chemotherapy. (22, 28-32) The two agents that are approved for this patient population are atezolizumab and nivolumab. Atezolizumab was the first such agent approved. It was evaluated in a phase II trial consisting of 310 patients with metastatic UCC progressing during or after a cisplatin based regimen. The ORR was 15%. The median PFS was 2.1 months and the median OS was 7.9 months for the whole study population. The median OS in those with PD-L1 staining by immunohistochemistry (IHC) of ≥ 5% on immune cells was 11.4 months. The vast majority of responses were still ongoing at data cutoff. These outcomes were felt to be comparable or better to second line chemotherapy with less toxicity. (22) A subsequent randomized phase III study demonstrated improved OS for pembrolizumab when compared to single agent chemotherapy for patients with advanced UCC after progression on platinum based chemotherapy. (33) Data for the trials of other ICI drugs in this patient population can be found in Table 2. (28-32)

TABLE 2: Immune Checkpoint Inhibitor Trials in Advanced Urothelial Cancer Patients Progressing During or After Prior Platinum Based Chemotherapy

Drug	**Target**	**Patients**	**Follow-up (m)**	**Phase**	**ORR by RECIST**	**PFS**	**OS**	**High Grade AEs**
Avelumab (PD-L1+ ≥ 5% on tumor cells)	PD-L1	44	13	Ib	18.2% (50% in PD-L1+ and 4.3% in PD-L1-)	24 weeks: 58.3% in PD-L1+, 16.6% PD-L1-	1 year 50.9%	11.3%
Durvalumab (PD-L1+ ≥ 25% on tumor cells or immune cells)	PD-L1	103	7.3	I/II	20.4% (29.5% in PD-L1+ and 7.7% in PD-L1-)	N/A	6 month OS 60.3% (PD-L1+	5.2%

							68.4%, PD-L1- 44.7%)	
Pembrolizumab (PD-L1+ ≥ 1% on tumor cells or immune cells)	PD-1	33	13	Ib	25% (38% in PD-L1+)	1 year 19%	N/A	15%
Nivolumab (PD-L1+ ≥ 1% on tumor cells)	PD-1	78	15.2	I/II	24.4% (24% PD-L1+and 26% PD-L1-)	Median 2.8 m, (PD-L1+ 5.5m, PD-L1- 2.8 m), 1 year PFS 21%	Median 9.7 m, (PD-L1+ 16.2m, PD-L1- 9.9 m), 1 year OS 46%	22%

m = months, ORR = overall response rate, PFS = progression free survival, OS = overall survival, AEs = adverse events, PD-1 = programmed death 1, PD-L1 = programmed death ligand 1, N/A = not available. High grade AEs are defined as ≥ 3 on the CTCAE grading scale.

Atezolizumab has data reported from the first-line setting for a cohort of patients with metastatic UCC who were ineligible for cisplatin-based combination chemotherapy. When results of this cohort were reported, the ORR was 23% and the median OS was 15.9 months. This OS was much higher than historical controls receiving carboplatin based regimens or single agent regimens, which has been reported at 9-10 months. (34)

ICI combinations have also been evaluated in metastatic UCC. While the full manuscript has not been published, the data has been presented in abstract form at the 2016 Society for Immunotherapy in Cancer (SITC) Annual Meeting. The combination of ipilimumab 3 mg/kg + nivolumab 1 mg/kg q 3 weeks x 4 cycles followed by nivolumab maintenance at 3 mg/kg q 2 weeks suggested an improved ORR compared to nivolumab monotherapy (38% vs 24%). (35)

The above studies which involve ICIs after progression despite platinum based chemotherapy have shown comparable if not better outcomes than historical controls given single agent chemotherapy (which the vast majority of patients receive) or combination therapy (only received by a select few). (36-38)

1.4 Cyclooxygenase Levels in Tumor Tissue

Prostaglandin E2 (PGE2) has been shown to increase production of tumor promoting factors (IL-6, IL-8 and Cxcl1) and decrease production of anti-tumor factors (IFN-y, IL-12, TNF, Cxcl9, Cxcl10, Cxcl11, CD8, CD40, CD86, antigen presenting dendritic cells and interferon response genes). Decreased PGE2 production via cyclooxygenase-1 and -2 (COX-1 and -2) inhibition has been shown to have an anti-tumor effect that is dependent on anti-tumor lymphocytes/dendritic cells. COX-1 and -2 inhibition lead to decreased tumor growth in mouse models of melanoma, colorectal and breast cancer with over-activation of the RAS/RAF/MEK pathway. The beneficial effect of COX-1 and -2 inhibition was lost when mice were depleted of dendritic cells and T/B

cells. Interestingly, the combination of aspirin (an irreversible COX-1 and -2 inhibitor) in combination with PD-1 inhibition led to significantly more suppression of tumor growth when compared to either monotherapy alone. There was no benefit of this combination in mice depleted of T and B cells. (39)

BC is a tumor type that may overexpress COX-1 and -2 enzymes. (40) COX-2 dependent production of PGE2 has a multifaceted role in mediating immunosuppression through induction of T regulatory cells and MDSCs, suppressing the accumulation of antigen-presenting dendritic cells, and most recently, as an inhibitory DAMP. (39, 41-43) Thus, we plan to examine mRNA levels COX-1 and COX-2 in pre-treatment biopsy specimens to determine if their levels may correlate with response or lack thereof to PD-L1 blockade.

1.5 Antitumor and Tumor Promoting Factors

Table 3: Mediators of an Effective Antitumor Immune Response

Tumor Promoting Chemokines/Cytokines and Enzymes	Growth Factors or Their Receptors that May Inhibit the Antitumor Immune Response	Cell Types that May Inhibit the Antitumor Immune Response	Anti-tumor Chemokines/Cytokines
Arginase-1, CCL2, CXCL8, CXCL12, IL-1, IL-6, , IDO, iNOS	M-CSFR, TGF-β, VEGF	MDSCs, T-regulatory cells (FOXP3+), M2 macrophages	CXCL9, CXCL10, IFN-γ, IL-2, IL-10*, IL-12

CCL = C-C motif chemokine ligand, CXCL = C-X-C motif chemokine ligand, IL = interleukin, IDO = indoleamine 2-3 dioxygenase, iNOS = inducible nitric oxide synthase, M-CSFR = macrophage colony stimulating factor receptor, TGF-β = transforming growth factor β, VEGF = vascular endothelial growth factor, MDSCs = myeloid derived suppressor cells, FOXP3 = forkhead box P3 and IFN-γ = interferon γ. *IL-10 was previously felt to be associated with inhibiting antitumor immunity; however, recent evidence suggests that it may induce an antitumor immune response in some tumors. (39, 44-47)

1.6 Avelumab Background

1.6.1 Structure, Mechanism and Identifiers

Avelumab is a fully human monoclonal PD-L1 antibody of the immunoglobulin G1 (IgG1) subclass. It works by binding to PD-L1 on tumor cells, immune cells and/or stromal cells. This prevents PD-L1 from interacting with PD-1. Inhibition of this interaction increases activation/survival of antitumor lymphocytes. It also increases innate immunity by resulting in decreased PD-1 suppression of NK cell function and bolsters antibody production by B cells due to less PD-L1 binding of PD-1 on B-cells. Additionally, avelumab has been suggested to have another mechanism involving antibody depemetandent cellular cytotoxicity (ADCC). ADCC in these cases involves NK cell recognition and lysis of tumor cells that have antibody bound to PD-L1. (48) By blocking PD-L1, avelumab leads to less CD80 binding by PD-L1 and more CD80-CD28 binding in response to antigen presentation to T-cells. This results in increased costimulatory signaling and is another mechanism by which avelumab may enhance T-cell activation. (49)

CAS Number: 1537032-82-8
UNII: KXG2PJ551I

1.6.2 Summary of non-clinical experience

The PD-1 pathway has been shown to be involved in inhibiting over activation of the immune system. This was supported by data in PD-1 deficient mice that showed development of delayed onset autoimmunity that was organ specific and had incomplete penetrance. After approximately 6 months C57BL/6 mice deficient in PD-1 developed a lupus like syndrome characterized by glomerulonephritis and arthritis. PD-1 deficient BALB/c mice developed an autoimmune dilated cardiomyopathy caused by antibodies to troponin-I. Selective deficiency of PD-L1 in mice may also result in autoimmune adverse events. (49)

Blockade of PD-1 or PD-L1 in mice did not have significant immune mediated adverse events in preclinical studies. However, these studies did suggest that such blockade may exacerbate pre-existing autoimmune pathology (e.g., significantly worsening autoimmune attack on the pancreas in a mouse with type I diabetes mellitus). (49)

PD-1 has been found to be highly expressed on T-cells in states of chronic viral infection. Blockade of PD-L1 on virally infected cells enhanced T-cell control of viruses. However, lack of expression of PD-L1 in mice resulted in fatal autoimmune pathology in the setting of chronic viral infection. Because of this there is uncertainty about the safety of blocking the PD-1 pathway in organisms with chronic viral infection. (49)

The epitope on PD-L1 that binds avelumab overlaps with the same epitope region that binds PD-1. This supports the mechanism of avelumab functioning by inhibiting interaction between PD-L1 and PD-1. (50) The other mechanism through which avelumab functions is by stimulating ADCC. This mechanism has been shown to be effective across multiple tumor types. (48, 51) PD-L1 inhibition by avelumab may also increase the availability of the co-stimulatory molecule CD80, allowing for enhanced activation of T-cells, through increased CD80-CD28 binding when antigens are presented by major histocompatibility (MHC) molecules to the T-cell receptor. (49) In vitro analysis of virus stimulated mononuclear cells from healthy patients have suggested that the mechanisms of avelumab lead to increased antigen specific CD8+ T-cells, reduced proliferation of CD4+ T-cells and a shift in the cytokine profile from an tumor promoting Th2 phenotype to a antitumor Th1 phenotype. (52)

The role of PD-L1 in impairing antitumor immunity has been supported in a variety of tumor models. PD-L1 expression on mastocytoma tumors was shown in vitro to promote immune evasion and enhance tumor growth. Blockade of PD-L1 on these same tumors promoted immune mediated rejection. Additionally, PD-L1 blockade on myeloma, melanoma and breast tumors has been shown to promote immune mediated tumor rejection. (49)

Avelumab has been examined in an orthotopic mouse model of NMIBC. In this model, avelumab had significant antitumor effects that appeared more mediated by CD4+ T-cells than CD8+ T-cells. (53)

1.6.3 Summary of Clinical Experience

Avelumab has been shown to be efficacious across multiple metastatic tumor types (Table 4). (54-59) One of these tumor types was UCC. There was a phase Ib study reported at the American Society of Clinical Oncology meeting in 2016 where data were presented on the

safety and efficacy of avelumab in the second line setting for metastatic UCC. Patients had been treated with a median of 2 prior therapies in the metastatic setting. High grade adverse events were 11.2%. The ORR was 18.2% and the 1 year OS was 50.9%. The ORR was better or comparable to chemotherapy in historical controls and the OS was improved compared to most other trials of second line or greater chemotherapy for such patients. There is currently a phase III clinical trial ongoing comparing avelumab to standard of care chemotherapy in the second line setting or beyond for metastatic UC. (28)

Table 4: Studies of Avelumab in Advanced Solid Tumors

Tumor Type	Phase	ORR by RECIST v1.1	PFS (median)	OS (median)	High Grade AEs
NSCLC after progression on platinum based chemo (n = 184)	Ib	Adeno – 11.4% SC – 13.2% Other – 29.4% (15% PD-L1+, PD-L1-10%)	11.6 wks (12 wks PD-L1+, 5.9 wks PD-L1-)	8.4 m (8.9 m PD-L1+, 4.6m PD-L1-)	12.5%
Urothelial cell carcinoma after progression on platinum based chemo (n = 44)	Ib	18.2% (50% in PD-L1+ and 4.3% in PD-L1-)	24 wks: 58.3% in PD-L1+, 16.6% PD-L1-	1 year 50.9%	11.3%
Breast Cancer unselected by subtype progressing on standard of care therapy (n = 168)	Ib	4.5% (33.3% PD-L1+, 2.4% PD-L1-, 8.8% TNBC)	N/A	N/A	14.3%
Merkel cell that was chemo refractory (n = 88)	II	31.8% (34.5% PD-L1+, 18.8% PD-L1-)	2.7 m, 40% was 6 m PFS	11.3 m, 69% was 6 m OS	5%
Ovarian cancer that was recurrent or refractory (n = 124)	Ib	9.7% (12.3% PD-L1+, 5.9% PD-L1-)	11.3 wks	10.8 m	6.5%
Mesothelioma progressing after a platinum-pemetrexed regimen (n = 53)	Ib	9.4% (14.3% PD-L1+, 8.0% PD-L1-)	17.1 wks (17.1 wks in PD-L1+, 7.4 wks in PD-L1-) 38.4% was 24 wk PFS	N/A	7.5%
Gastric or GE junction cancer as maintenance after first line therapy or as second line treatment (n = 151)	Ib	Maintenance – 9.0% (10% PD-L1+, 3.1% PD-L1-) Second line – 9.7% (18.2% PD-L1+, PD-L1- 9.1%)	Maintenance – 12 wks (17.6 wks PD-L1+, 11.6 wks PD-L1-) Second line – 6 wks (6.3 wks PD-L1+, 10.4 wks PD-L1-)	N/A	9.9%

ORR=overall response rate, RECISTv1.1 = response evaluation criteria in solid tumors version 1.1, PFS = progression free survival, OS = overall survival, AEs = adverse events, NSCLC = non-small cell lung cancer, chemo

= chemotherapy, Adeno = adenocarcinoma, SC = squamous cell carcinoma, PD-L1 = programmed death ligand 1, wks = weeks, m = months, TNBC = triple negative breast cancer, N/A = not available, GE = gastroesophageal

1.7 Research Hypotheses

- Primary Hypothesis: Avelumab treatment will result in an increase in CD3+CD4+ T-cell and CD3+CD8+ T-cell infiltrates.

- Secondary Hypotheses:
    - Pathological response rate will be comparable to historical controls receiving NA cisplatin based chemotherapy.
    - 2 year disease free survival (DFS) will be better than historical controls not receiving NAC and comparable to historical controls receiving cisplatin based NAC.
    - Avelumab will be safe and tolerable in our patient population.
    - Infiltration of regulatory B-cells will increase with avelumab treatment and increased levels will be associated with less response to treatment.
    - Increased COX-1 and COX-2 levels on transurethral resection of the bladder tumor (TURBT) tissue will be associated with decreased response to avelumab.

1.8 Rationale for Conducting this Study

Up to 50% of patients are unable or unwilling to receive cisplatin-based NAC and their median DFS ranges from 12 to 48 months, compared to 48 months to > 5 years for those who receive cisplatin-based NAC. (60) Patients who are unable to receive cisplatin-based NAC go straight to RC. There is no approved NA systemic therapy for this population and survival outcomes are significantly inferior to those who receive cisplatin-based NAC. ICIs have been observed to be superior to non-cisplatin based chemotherapy in the first line setting for metastatic UCC and have shown significant efficacy after progression on platinum based chemotherapy in metastatic UCC. (28-34) Thus, there is rationale that ICIs may be of benefit in the NA treatment of MIBC patients who are not candidates for cisplatin-based NAC. We plan to examine this as one of our secondary endpoints in this window of opportunity trial.

1.9 Benefit/risk and Ethical Assessment

High grade (grade 3-4) AEs with PD-1 or PD-L1 inhibitors in metastatic UCC range from 5-22%, with such events being slightly lower in those who received PD-L1 as opposed to PD-1 inhibitory antibodies. (28-33) Our inclusion and exclusion criteria are in agreement with these previous studies and thus we expect to see a similar incidence of AEs. This estimated occurrence of high grade AEs is significantly less than the 50-80% seen with cisplatin-based NAC or with cisplatin-based chemotherapy in the metastatic setting. (11, 61-62)

We know that cisplatin-based NAC provides a significant survival benefit for non-metastatic MIBC and that ICIs are suggested to have similar or better efficacy than systemic chemotherapy in metastatic BC. Thus, the potential benefit to administering avelumab in the NA setting is significant. The benefit to risk ratio of this trial is judged to be acceptable.

1.9.1 Risks Associated with Avelumab

Three types of risks are associated with avelumab: general signs and symptoms, reactions that occur during or following the infusion, and immune side effects.

The following side effects have been observed among 1,738 patients treated with avelumab according to the results from two oncology clinical studies in patients with various solid tumors.

Side effects observed in 10% or more of patients:

- **General signs or symptoms**: Tiredness; Nausea; Loose or watery stools (diarrhea); Constipation; Reduced appetite; Decrease in weight; Vomiting; Low number of red blood cells (anemia); Belly pain; Cough; Fever; Shortness of breath; Swelling of feet and legs; Back pain; Joint pain.
- **Reactions that occur during or following the infusion:** may include chills or shaking, fever, flushing, back pain, belly pain, shortness of breath or wheezing, decrease in blood pressure, hives. These infusion reactions are mostly mild or moderate and generally resolve with a slowdown or discontinuation of the infusion and administration of medications such as anti-allergic and pain-killer drugs. In some cases these reactions may be severe or life-threatening (in less than 1% of patients) and can require intensive medical care.

Immune side effects

Immune side effects result from an increased activity of the immune system. Most of these side effects are reversible, which means they will stop once treatment with avelumab is discontinued. However, in some cases these reactions may be severe (approximately 2% of patients) and may lead to death in rare cases. The reactions that are more severe require treatment with drugs that decrease the immune system function, also called immunosuppressant drugs (like corticosteroids or more potent drugs). No immune side effects were observed in 10% or more of patients.

Immune side effects observed in 5% to less than 10% of patients:

- **Abnormal function of the thyroid gland (could include low or high function or inflammation of the thyroid gland):** may include rapid heartbeat; increased sweating; extreme tiredness; weight gain or weight loss; hair loss; changes in mood or behavior such as irritability or forgetfulness; feeling cold; constipation; voice gets deeper.
- **Inflammation of the skin (rash):** may include skin rash, itchy skin, skin redness, skin blisters, or peeling.

Immune side effects observed in 1% to less than 5% of patients:

- **Inflammation of the large intestine (colitis):** may include diarrhea (loose stools) or more frequent bowel movements than usual; blood in stools or dark, tarry, sticky stools; severe stomach area (abdomen) pain or tenderness.
- **Inflammation of the lungs (pneumonitis):** may include new or worsening cough, shortness of breath, chest pain.

Immune side observed in less than 1% of patients:

- **Inflammation of the liver (hepatitis):** may include yellowing of skin or of the whites of eyes; severe nausea or vomiting; pain on the right side of stomach area (abdomen); drowsiness; dark urine (tea colored); bleeding or bruising more easily than normal; feeling less hungry than usual.
- **Inflammation of the kidneys (nephritis):** may include urinating less than usual; blood in urine; swelling in ankles; loss of appetite.

- **Low function of the adrenal glands (glands on top of the kidneys), which may be due to the reduced function of the pituitary gland (a gland in the head):** may include very low blood pressure; extreme tiredness.
- **Increase in blood sugar (diabetes):** may include urinating more often than usual; feeling more hungry or thirsty than usual, nausea or vomiting, stomach area (abdomen) pain.
- **Inflammation of the eyes (uveitis):** may include changes in eyesight.
- **Inflammation of the muscles (myositis):** may include severe or persistent muscle or joint pain; severe muscle weakness.
- **Inflammation of the heart (myocarditis):** may include chest pain or tightness; tiredness; changes in heartbeat, such as beating fast, or seeming to skip a beat, or pounding sensation; swelling of feet and legs; trouble breathing.
- **Inflammation of the nerves (Guillain-Barre syndrome):** may include "pins and needles" sensations in arms and legs; weakness in legs that spreads to the upper body and may lead to temporary paralysis.

2. STUDY OBJECTIVES

2.1 Primary objective(s)

- To evaluate change in T cell subpopulations (CD8, CD4 and/or CD3) in tumor samples collected pre- and post-treatment with avelumab.

2.2 Secondary objective(s)

- To evaluate pathologic partial response (downstaging to < pT2) and complete response
- To evaluate 2 year DFS
- To evaluate safety of drug in the NA setting
- To evaluate correlation of translational studies with response parameters

2.3 Translational endpoints

- To evaluate PD-L1 by IHC and COX-1/COX-2 expression by mRNA in pre- treatment cancer tissues (focusing on epithelial cell expression and immune cell expression).
- To evaluate infiltrating CD3+, CD4+ and CD3+, CD8+ T cells in pre- and post- PD-L1 antibody treated patient cancer tissues by IHC.
- To evaluate change in regulatory B-cell populations with treatment as identified by IHC for CD1d, CD5, CD19 and CD24.
- To evaluate mRNA levels of the following chemokines/cytokines and immune checkpoints on formalin fixed paraffin embedded (FFPE) tissue from pre- and post-treatment specimens: IFN-γ, TNF-α, TNF-β, T-box protein in B-cells (T-bet), IL-2, IL-10, IL-35, FOXP-3, CTLA-4, LAG-3, PD-L1, Granzyme-A and Perforin-1.

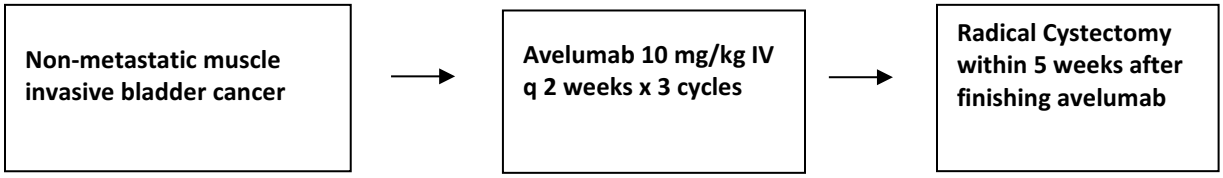
3. STUDY DESIGN

3.1 Overview of Study Design

This is a pilot study of avelumab in patients with non-metastatic, MIBC who are eligible for RC, but refuse or are ineligible for cisplatin based NAC. The target recruitment is 10 evaluable patients for this window of opportunity study. Pre- and post-treatment tumor samples from TURBT and RC will be used for study endpoints.

Patients will be recruited from sites within the academic system of Baylor College of Medicine. Recruitment will be over one year and patients will undergo systemic treatment followed by surgery as per protocol. They will be followed for 2 years for survival, recurrence, and safety monitoring.

3.2 Study Schema



3.3 Study Oversight for Safety Evaluation

In metastatic UCC, the high grade treatment related adverse event rate with avelumab is approximately 10% and the rate of serious adverse events (SAEs) is 5-10%. (54-59)

Treatment may be put on hold, and pending full review of the data, the study may be stopped if any of the following criteria are met:

- Two (2) avelumab-related SAEs are reported
- At least 2 subjects experience a similar unanticipated avelumab-related AE

3.4 Evaluability and Patient Replacement

Patients will continue to be enrolled on study until we have enrolled/treated 10 patients who are evaluable for the primary endpoint. Patients will be replaced after enrollment if they are later determined not to be evaluable for the primary endpoint.

<u>Evaluable patients</u>

- For primary endpoint
  - Receive ≥ 1 dose of avelumab, undergo RC within 5 weeks of last dose of avelumab, and are not started on systemic steroids at a dose of > 10 mg/d of prednisone or equivalent, while taking avelumab.
- For safety endpoint
  - Receive ≥ 1 dose of avelumab
- For pathologic complete response/partial response
  - Receive ≥ 1 dose of avelumab and undergo RC < 42 days from last avelumab dose
- For 2 year disease free survival
  - Meet evaluability criteria for complete response/partial response, participate in 90 day on study follow-up after RC and participate in off study follow-up as part of regular care up to 2 years post RC.

<u>Inevaluable patients</u>

- Patients who receive < 1 dose of avelumab after study enrollment for any reason.

4. SUBJECT SELECTION: PATIENT INITIALS: _____

4.1 Inclusion Criteria

____ a) Have undergone TURBT showing newly diagnosed muscle invasive UCB (mixed histology is allowed if the predominant histology is UCC) within 6 weeks prior to cycle 1, day 1 of treatment.

____ b) No prior systemic treatment for muscle invasive UCB

____ c) Clinical T2-T4a disease

____ d) No evidence of clinically positive lymph nodes or distant metastasis on computed tomography (CT) scans of chest and CT or magnetic resonance imaging (MRI) studies of the abdomen/pelvis. Imaging must be within 90 days of registration.

____ e) Male or female subjects aged ≥ 18 years old.

____ f) Must have adequate kidney, liver, and bone marrow function within 30 days of registration, as follows:

a. Absolute neutrophil count (ANC) ≥ 1.5×10^9/L

b. platelet count ≥ 100×10^9/L

c. hemoglobin ≥ 9 g/dL (may have been transfused)

d. Total bilirubin level ≤ 1.5 × ULN

e. AST and ALT levels ≤ 2.5 × ULN

f. Estimated creatinine clearance ≥ 30 mL/min according to the Cockcroft-Gault formula

____ g) Negative serum or urine pregnancy test at screening for women of childbearing potential (WOCBP), within 30 days of registration.

____ h) Both male and female subjects must agree to use highly effective contraception (see Section 6.1 Table 8) while receiving avelumab and for at least 60 days after last avelumab treatment if the risk of conception exists.

Female patients must agree to inform study coordinator or investigator immediately if they think they have become pregnant during the study.

____ i) Must have FFPE tissue available from the TURBT, and patient must consent to the use of tissue specimens from TURBT and RC for the study.

____ j) Patients must have refused or have been determined to be ineligible for cisplatin-based NAC. Ineligibility criteria include: creatinine clearance < 60 ml/min by Cockcroft-Gault formula, CTCAE grade ≥ 2 hearing loss, CTCAE grade ≥ 2 neuropathy, and at discretion of medical oncologist.

If a patient is eligible for cisplatin-based NAC but has refused it, the reason for refusal must be documented in the patient record.

____ k) Must be eligible for RC in the opinion of the treating investigator, and willing to undergo this procedure.

____ l) ECOG performance status (PS) score of 0-2

____ m) Signed informed consent form.

4.2 Exclusion Criteria

Patients must not have any of the following:

____ a) IMMUNOSUPRESSANTS: Current use of immunosuppressive medication or within 4 weeks of C1D1, EXCEPT for the following: a. intranasal, inhaled, topical steroids, or local steroid injection (e.g., intra-articular injection); b. Systemic corticosteroids at physiologic doses ≤ 10 mg/day of prednisone or equivalent; c. Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication).

____ b) AUTOIMMUNE DISEASE: Active autoimmune disease that might deteriorate when receiving an immuno-stimulatory agent. Patients with diabetes type I, vitiligo, psoriasis, or hypo- or hyperthyroid diseases not requiring immunosuppressive treatment are eligible. Patients with type I diabetes or hypo- or hyperthyroidism should be on stable doses of medications for participation.

____ c) ORGAN TRANSPLANTATION: Prior organ transplantation including allogenic stem-cell transplantation.

____ d) INFECTIONS: Active infection requiring systemic therapy.

____ e) HIV/AIDS: Known history of testing positive for HIV or known acquired immunodeficiency syndrome.

____ f) HEPATITIS: Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection at screening (positive HBV surface antigen or HCV RNA if anti-HCV antibody screening test positive)

____ g) VACCINATION: Vaccinate within 4 weeks of the first dose of avelumab and while on study drug is prohibited except for administration of inactivated vaccines

____ h) HYPERSENSITIIVTY TO STUDY DRUG: Known prior severe hypersensitivity to investigational product or any component in its formulations, including known severe hypersensitivity reactions to monoclonal antibodies (NCI CTCAE v4.03 Grade ≥ 3)

____ i) CARDIOVASCULAR DISEASE: Clinically significant (i.e., active) cardiovascular disease: cerebral vascular accident/stroke (< 6 months prior to enrollment), myocardial infarction (< 6 months prior to enrollment), unstable angina, congestive heart failure (≥ New York Heart Association Classification Class II), or serious cardiac arrhythmia requiring medication.

____ j) Other severe acute or chronic medical conditions including colitis, inflammatory bowel disease, pneumonitis, pulmonary fibrosis or psychiatric conditions including recent (within the past year) or active suicidal ideation or behavior; or laboratory abnormalities that may increase the risk associated with study participation or study treatment administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for entry into this study.

____ k) Relapsed MIBC (all patients participating in the study should be newly diagnosed)

____ l) Concomitant UCC outside the bladder (e.g., ureter, urethra or renal pelvis)

____ m) Underlying immune disorder (e.g., combined variable immunodeficiency syndrome)

____ n) Erythropoietin receptor agonists within 30 days prior to enrollment.

____ o) G-CSF, GM-CSF or TPO mimetics during the study period or within 3 weeks prior to study enrollment

____ p) Malignancies other than UCB within 5 years prior to Cycle 1, Day 1, with the exception of those with low risk of metastasis or death treated with expected curative outcome (such as, but not limited to, adequately treated carcinoma in situ of the cervix, basal or squamous cell skin cancer, localized prostate cancer treated with curative intent and absence of PSA relapse, or ductal carcinoma in situ of the breast treated surgically with curative intent) or incidental prostate cancer (T1a, Gleason score ≤ 6 and PSA < 0.5 ng/ml)

____ q) Prior immunotherapy with T-cell co-stimulation or checkpoint targeted agents (e.g., CTLA-4 inhibitors, anti-PD1 antibodies or anti-PD-L1 antibodies)

____ r) Intravesical chemotherapy or biologic therapy within 6 weeks of Cycle 1, Day 1

____ s) Current participation in another clinical trial for MIBC

____ t) Nursing or pregnant woman

____ u) Uncontrolled cystitis, significant bladder pain or spasms, or gross hematuria that in the opinion of the principal investigator will preclude study participation

____ v) Major surgical procedures within 4 weeks of registration (other than for diagnosis) or anticipation that such a procedure will be needed during the study (other than RC)

Treating Investigator Name: _____

Treating Investigator Signature: _____

Date: _____

4.3 Withdrawal of Subjects from Study Treatment and/or Study

An individual subject will not receive any further investigational product if any of the following occur after enrollment on study:

- Withdrawal of consent by subject
- Subject is determined to have met one or more of the exclusion criteria for study participation at study entry and continuing investigational therapy might constitute a safety risk
- Pregnancy or intent to become pregnant
- Any criteria that necessitates discontinuation of study treatment as outlined in sections 5.5-5.7
- Subject noncompliance that in the opinion of the investigator or sponsor warrants withdrawal; e.g., refusal to adhere to scheduled visits
- Initiation of alternative anticancer therapy including another investigational agent
- Subject has > 2 week delay in the administration of avelumab. Such patients will still receive surgery and laboratory/clinical follow-up to complete the 90 day safety monitoring after RC. Some of these patients may be evaluable for primary and/or secondary outcomes.

Subjects who are permanently discontinued from further receipt of avelumab, regardless of the reason, will be identified as having permanently discontinued treatment.

- Withdrawal of consent
  Patients are free to withdraw their consent to further participation in this trial without prejudice. If consent is withdrawn, the subject will not receive any further investigational product.

- Withdrawal from study
  Patients determined to be inevaluable will be removed from study. Such patients will receive standard of care and follow-up. Such patients will be followed off study for recurrence and/or survival as part of routine care.

5. TREATMENT PLAN

5.1 Premedication

The following will be administered 30-60 minutes prior to avelumab

- Diphenhydramine: 25 mg IV, or 25-50 mg PO
- Paracetamol (acetaminophen): 500 to 650 mg PO

5.2 Dosage and Administration of Avelumab

- Avelumab will be given at a dose of 10 mg/kg IV over 60 minutes every 2 weeks (Days 1, 15, and 29), for 3 cycles. Each cycle is 14 days.

- Drug will be given in outpatient infusion centers, all of which have immediate access to emergency services or critical care. The following medications or equivalents will be immediately available in the case of a severe allergic reaction: 10 mg dexamethasone, epinephrine (1:1000 dilution), IV anti-histamines, bronchodilators and oxygen.

- Patients will be observed for 2 hours post-infusion to monitor for infusion reactions.

5.3 Radical Cystectomy (RC)

- Patients will undergo RC within five weeks after the 3rd dose of avelumab.
- Cystectomy will be done per standard clinical care.

5.4 Supportive Care

- Patients will receive supportive care for acute or chronic toxicity, including blood components or antibiotics, and other intervention as appropriate

5.5 Table 5: Adverse Drug Reactions Requiring Avelumab Discontinuation or Modification

Any Grade 4 ADR requires treatment discontinuation with avelumab, except for single laboratory values out of normal range that are unlikely related to study treatment as assessed by the Investigator, do not have any clinical correlate, and resolve within 7 days with adequate medical management.

Any Grade 3 ADR requires treatment discontinuation with avelumab except for any of the following:

- Transient (≤ 6 hours) Grade 3 flu-like symptoms or fever, which is controlled with medical management
- Transient (≤ 24 hours) Grade 3 fatigue, local reactions, headache, nausea, emesis that resolves to Grade ≤ 1
- Single laboratory values out of normal range (excluding Grade ≥ 3 liver function test increase) that are unlikely related to study treatment according to the Investigator, do not have any clinical correlate, and resolve to Grade ≤ 1 within 7 days with adequate medical management
- Tumor flare phenomenon defined as local pain, irritation, or rash localized at sites of known or suspected tumor

- Change in ECOG PS to ≥ 3 that resolves to ≤ 2 within 14 days (infusions should not be given on the following cycle, if the ECOG PS is ≥ 3 on the day of study drug administration)

Any Grade 2 ADR should be managed as follows:

- If a Grade 2 ADR resolves to Grade ≤ 1 by the last day of the current cycle, treatment may continue.
- If a Grade 2 ADR does not resolve to Grade ≤ 1 by the last day of the current cycle, infusions should not be given on the following cycle. If at the end of the following cycle the event has not resolved to Grade 1, the subject should permanently discontinue treatment with avelumab ADR (except for hormone insufficiencies that can be managed by replacement therapy.
- Upon the second occurrence of the same Grade 2 ADR (except for hormone insufficiencies that can be managed by replacement therapy) in the same subject, treatment with avelumab has to be permanently discontinued.

5.6 Table 6: Treatment Modification for Symptoms of Infusion-Related Reactions

NCI-CTCAE Grade	Modification of Study Drug
Grade 1 – mild Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Decrease the study drug infusion rate by 50% and monitor closely for any worsening. The total infusion time for study drug should not exceed 120 minutes.
Grade 2 – moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (for example, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 h.	Hold the study drug infusion. Resume infusion at 50% of previous rate once infusion-related reaction has resolved or decreased to ≤ Grade 1 in severity, and monitor closely for any worsening.
Grade 3 or Grade 4 – severe or life-threatening Grade 3: Prolonged (for example, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae. Grade 4: Life-threatening consequences; urgent intervention indicated.	Stop the study drug infusion immediately and disconnect infusion tubing from the subject. Subjects have to be withdrawn immediately from study drug treatment and must not receive any further study drug treatment.
- Once the avelumab infusion rate has been decreased by 50% or interrupted due to an infusion-related reaction, it must remain decreased for all subsequent infusions. - If the subject has a second infusion-related reaction Grade ≥ 2 on the slower infusion rate, the infusion should be stopped and the subject should be removed from study treatment. - If hypersensitivity reaction occurs, the subject must be treated according to the best available medical practice.	

IV = intravenous; NCI-CTCAE = National Cancer Institute-Common Terminology Criteria for Adverse Event; NSAIDs = nonsteroidal anti-inflammatory drugs.

5.7 Table 7: Management of Immune-Related Adverse Reactions

Since inhibition of PD-L1 stimulates the immune system, immune-related AEs (irAEs) may occur. Treatment of irAEs is mainly dependent upon severity (NCI-CTCAE grade):
- Grade 1 to 2: treat symptomatically or with moderate dose steroids, more frequent monitoring
- Grade 1 to 2 (persistent): manage similar to high grade AE (Grade 3 to 4)
- Grade 3 to 4: treat with high dose corticosteroids

Treatment of gastrointestinal, dermatological, pulmonary, hepatic, endocrine and cardiac irAEs should follow guidelines set forth in the table below.

Gastrointestinal irAEs		
Severity of Diarrhea / Colitis (NCI-CTCAE v4.03)	**Management**	**Follow-up**
Grade 1 Diarrhea: < 4 stools/day over baseline Colitis: asymptomatic	Continue avelumab therapy Symptomatic treatment (for example, loperamide)	Close monitoring for worsening symptoms Educate subject to report worsening immediately If worsens: Treat as Grade 2 or 3/4
Grade 2 Diarrhea: 4 to 6 stools per day over baseline; IV fluids indicated < 24 hours; not interfering with ADL Colitis: abdominal pain; blood in stool	Delay avelumab therapy unless systemic steroids needed. If systemic steroids are needed, discontinue avelumab. Symptomatic treatment	If improves to Grade 1: Resume avelumab therapy If persists > 5 to 7 days or recurs: 0.5 to 1.0 mg/kg/day methylprednisolone or equivalent; when symptoms improve to Grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections and remove patient from study treatment. If worsens or persists > 3 to 5 days with oral steroids: Treat as Grade 3 to 4
Grade 3 to 4 Diarrhea (Grade 3): ≥ 7 stools per day over baseline; incontinence; IV fluids ≥ 24 hrs.; interfering with ADL	Discontinue avelumab therapy per protocol 1.0 to 2.0 mg/kg/day methylprednisolone IV or equivalent	If improves: Continue steroids until Grade 1, then taper over at least 1 month If persists > 3 to 5 days, or recurs after improvement:

Colitis (Grade 3): severe abdominal pain, medical intervention indicated, peritoneal signs Grade 4: life-threatening, perforation	Add prophylactic antibiotics for opportunistic infections Consider lower endoscopy	Add infliximab 5 mg/kg (if no contraindication), Note: Infliximab should not be used in cases of perforation or sepsis
Dermatological irAEs		
Grade of Rash (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1 to 2 Covering ≤ 30% body surface area	Symptomatic therapy (for example, antihistamines, topical steroids) Continue avelumab therapy unless systemic steroids are needed. If systemic steroids are needed, discontinue avelumab.	If persists > 1 to 2 weeks or recurs: Consider skin biopsy Delay avelumab therapy Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and if systemic steroids used discontinue study treatment. If worsens: Treat as Grade 3 to 4
Grade 3 to 4 Covering > 30% body surface area; life threatening consequences	Discontinue avelumab therapy Consider skin biopsy Dermatology consult 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent	If improves to Grade 1: Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections
Pulmonary irAEs		
Grade of Pneumonitis (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1 Radiographic changes only	Consider delay of avelumab therapy	Re-image at least every 3 weeks

	Monitor for symptoms every 2 to 3 days Consider Pulmonary and Infectious Disease consults	If worsens: Treat as Grade 2 or Grade 3 to 4
Grade 2 Mild to moderate new symptoms	Discontinue avelumab therapy Pulmonary and Infectious Disease consults Monitor symptoms daily, consider hospitalization 1.0 mg/kg/day methyl-prednisolone IV or oral equivalent Consider bronchoscopy, lung biopsy	Re-image every 1 to 3 days If improves: When symptoms return to near baseline, taper steroids over at least 1 month and consider prophylactic antibiotics. If not improving after 2 weeks or worsening: Treat as Grade 3 to 4
Grade 3 to 4 Severe new symptoms; New / worsening hypoxia; life-threatening	Discontinue avelumab therapy Hospitalize Pulmonary and Infectious Disease consults 2 to 4 mg/kg/day methylprednisolone IV or IV equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	If improves to baseline: Taper steroids over at least 6 weeks If not improving after 48 hours or worsening: Add additional immunosuppression (for example, infliximab, cyclophosphamide, IV immunoglobulin, or mycophenolate mofetil)

Hepatic irAEs		
Grade of Liver Test Elevation (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1 AST or ALT > ULN to 3.0 x ULN and / or total bilirubin > ULN to 1.5 x ULN	Continue avelumab therapy	Continue liver function monitoring If worsens: Treat as Grade 2 or 3 to 4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and / or total bilirubin > 1.5 to ≤ 3 x ULN	Delay avelumab therapy. If systemic steroids are required, avelumab therapy will be discontinued. Increase frequency of monitoring to every 3 days	If returns to Baseline: Resume routine monitoring, resume avelumab therapy If elevations persist > 5 to 7 days or worsens: 0.5 to 1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and discontinue avelumab therapy

Grade 3 to 4 AST or ALT > 5 x ULN and / or total bilirubin > 3 x ULN	Discontinue avelumab therapy Increase frequency of monitoring to every 1 to 2 days 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent Add prophylactic antibiotics for opportunistic infections Consult gastroenterologist Consider obtaining MRI/CT scan of liver and liver biopsy if clinically warranted	If returns to Grade 2: Taper steroids over at least 1 month If does not improve in > 3 to 5 days, worsens or rebounds: Add mycophenolate mofetil 1 gram (g) twice daily If no response within an additional 3 to 5 days, consider other immunosuppressants per local guidelines
Endocrine irAEs		
Endocrine Disorder	**Management**	**Follow-up**
Asymptomatic TSH abnormality	Continue avelumab therapy If TSH < 0.5 x LLN, or TSH > 2 x ULN, or consistently out of range in 2 subsequent measurements: include T4 at subsequent cycles as clinically indicated; consider endocrinology consult	
Symptomatic endocrinopathy	Evaluate endocrine function Consider pituitary scan Symptomatic with abnormal lab / pituitary scan: Discontinue avelumab therapy 1 to 2 mg/kg/day methylprednisolone IV or by mouth equivalent (with the exception of diabetes mellitus)	If improves (with or without hormone replacement): Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections Subjects with adrenal insufficiency may need to continue steroids with mineralocorticoid component

	Initiate appropriate hormone therapy No abnormal lab / pituitary MRI scan but symptoms persist: Repeat labs in 1 to 3 weeks / MRI in 1 month	
Suspicion of adrenal crisis (for example, severe dehydration, hypotension, shock out of proportion to current illness)	Delay or discontinue avelumab therapy Rule out sepsis Stress dose of IV steroids with mineralocorticoid activity IV fluids Consult endocrinologist If adrenal crisis ruled out, then treat as above for symptomatic endocrinopathy.	
Cardiac irAEs		
Myocarditis	**Management**	**Follow-up**
New onset of cardiac signs or symptoms and / or new laboratory cardiac biomarker elevations (e.g. troponin, CK-MB, BNP) or cardiac imaging abnormalities suggestive of myocarditis.	Withhold avelumab therapy Hospitalize. In the presence of life threatening cardiac decompensation, consider transfer to a facility experienced in advanced heart failure and arrhythmia management. Cardiology consult to establish etiology and rule-out immune-mediated myocarditis. Guideline based supportive treatment as appropriate per cardiology consult.* Consider myocardial biopsy if recommended per cardiology consult.	If symptoms improve and immune-mediated etiology is ruled out, re-start avelumab therapy. If symptoms do not improve/worsen, viral myocarditis is excluded, and immune-mediated etiology is suspected or confirmed following cardiology consult, manage as immune-mediated myocarditis.
Immune-mediated myocarditis	Permanently discontinue avelumab. Guideline based supportive treatment as appropriate per cardiology consult.*	Once improving, taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections.

	Methylprednisolone 1-2 mg/kg/day.	If no improvement or worsening, consider additional immunosuppression (e.g. azathioprine, cyclosporine A)

ADL = activities of daily living; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CT = computed tomography; irAE = immune-related adverse event; IV=intravenous; LFT = liver function test; LLN = lower limit of normal; MRI = magnetic resonance imaging; NCI-CTCAE = National Cancer Institute-Common Terminology Criteria for Adverse Event; T4 = free thyroxine; TSH = thyroid-stimulating hormone; ULN = upper limit of normal. *Local guidelines, or eg. ESC or AHA guidelines. ESC guidelines website: https://www.escardio.org/Guidelines/Clinical-Practice-Guidelines, AHA guidelines website: http://professional.heart.org/professional/GuidelinesStatements/searchresults.jsp?q=&y=&t=1001

6. RESTRICTIONS DURING THE STUDY AND CONCOMITANT TREATMENTS

6.1 Restrictions during the study

Contraception
Women of childbearing potential (WOCBP) who are sexually active with a non-sterilized male partner must use at least one highly effective method of contraception (Table 8) from the time of screening and must agree to continue using such precautions for 60 days after the last dose of avelumab. The male partner of a female subject must also use male condom plus spermicide throughout this period. Cessation of birth control after this point should be discussed with a responsible physician. Not engaging in sexual activity is an acceptable practice; however, occasional abstinence, the rhythm method, and the withdrawal method are not acceptable methods of contraception. Female patients should refrain from breastfeeding and egg cell donation throughout this period.

- Females of childbearing potential are defined as those who are not surgically sterile (i.e., bilateral tubal ligation, bilateral oophorectomy, or complete hysterectomy) or postmenopausal (defined as 12 months with no menses without an alternative medical cause).
- Effective methods (including highly effective methods) of contraception are described in Table 8. A highly effective method of contraception is defined as one that results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly. Note that not all methods in Table 8 are considered highly effective.
- Non-sterilized male subjects who are sexually active with a female partner of childbearing potential must use male condom plus spermicide (see Table 8) from screening through 60 days after the last dose of avelumab. Not engaging in sexual activity is an acceptable practice; however, occasional abstinence, the rhythm method, and the withdrawal method are not acceptable methods of contraception. Male patients should refrain from sperm donation throughout this period. Female partners of a male subject must use an effective method of contraception throughout this period.

Table 8: Effective Methods of Contraception

Barrier/Intrauterine Methods	Hormonal Methods
• Male or female condom with or without spermicide [a,b,c] • Female cap, diaphragm or sponge with spermicide [a,b,c] • Copper T intrauterine device [e] • Levonorgesterel-releasing intrauterine system (eg, Mirena®) [d,e]	• Implants[e] • Hormone shot or injection[e] • Combined pill[e] • Minipill[b] • Patch[e]

[a] Female partners of male subjects must use an effective method of birth control
[b] Not highly effective (i.e. failure rate of >1% per year)
[c] A combination of male condom with either cap, diaphragm or sponge with spermicide (double barrier methods) are also considered acceptable, but not highly effective, birth control methods
[d] This is also considered a hormonal method
[e] Highly effective (i.e. failure rate of <1% per year)

Blood donation
Subjects should not donate blood while participating in this study, and for at least 60 days following the last infusion of avelumab.

6.2 Excluded concomitant medications or therapies

The following medications are considered exclusionary during the study.

- Any investigational anticancer therapy other than avelumab.
- Any concurrent chemotherapy, radiotherapy, immunotherapy, biologic or hormonal therapy for cancer treatment.
- Growth factors (including G-CSF, GM-CSF, EPO agonists or TPO mimetics)
- Systemic administration of immunosuppressive medications will not be allowed except for those detailed in section 4.2 and those used to manage adverse events as detailed in sections 5.5-5.7 of the protocol.
- Live vaccination within 4 weeks of the first dose of avelumab and while on trial is prohibited. Inactivated vaccines are allowed to be administered.

7. STUDY PROCEDURES

7.1 Study Calendar

Time Points	Pre-study	C1	C2	C3	Pre-surgery[b]	Surgery	Follow-up			End of Study visit	Follow-up
	30 days before D1	D1	D15	D29	D40-50	D41-64	2-3 weeks post-op	30 days post-op	60 days post-op	90 days post-op[c]	To 2 years post-op
Study Assessment											
Signed Informed Consent	X										
Eligibility Criteria	X										
Cancer Staging	X										
Physical Exam with ECOG Performance Status (PS)	X[a]		X	X	X[b]		X	X	X	X[c]	
Medical History/Smoking History/Occupation Hazard	X										
FFPE Tissue for Translational Endpoints[d]	X						X				
Laboratory Analysis											
CBC with diff and CMP	X		X	X	X		X	X	X	X	
UPT (if female of CBP)	X			X	X						
Hepatitis B surface antigen, Hepatitis C antibody and HIV 1/2 ELISA	X										
Hepatitis C RNA (if hepatitis C antibody positive)	X										
ACTH, Hemoglobin A1C, and TSH	X				X			X		X	
Free T4 (if TSH abnormal as indicated in Table 7)	X				X			X		X	
Imaging											
CT chest[e]	X				X					X	
CT or MRI abdomen/pelvis[f]	X				X					X	
Treatment											
Avelumab		X	X	X							
Adverse events and their management noted			X	X	X		X	X	X	X[g]	
Radical Cystectomy						X					
Pathology Assessment							X				
DFS Follow-up post-study											X

Footnotes:

a. Enrollment history and physical.
b. End of treatment visit.
c. End of study visit to be performed no earlier than Day 90 after RC.
d. FFPE tissue from the pre-study time point refers to tissue from the TURBT. FFPE tissue from the 2-3 week post-op time point refers to FFPE tissue from the RC.
e. Contrast will be used as long as not precluded by creatinine clearance. If a patient is allergic to CT contrast they will be appropriately pre-medicated with steroids +/- antihistamines unless they have a history of anaphylaxis. CT of the chest to be completed in +/- 14 days.
f. CT with contrast is preferable. However, MRI with contrast or non-contrast CT may be done based on creatinine clearance, allergies, and ability to tolerate contrast. If a patient is allergic to CT contrast, they will be appropriately pre-medicated with steroids +/- antihistamines unless they have a history of anaphylaxis. CT of the abdomen/pelvis to be completed in +/- 14 days.
g. To be performed at the end of study visit.

Allowable Windows:

1. TURBT should be done within 6 weeks of registration.
2. Avelumab administration should be within +/- 3 days of listed days.
3. Laboratory analysis on day 15 and day 29 may be performed on the day of infusion if results are received prior to avelumab administration. Alternatively, these laboratory analysis may be performed the day prior to avelumab administration.
4. The **end of treatment visit** (post-treatment pre-surgery) should be performed between days 40 and 50.
5. The **end of study visit** should be 90 days after surgery +/- 10 days.
6. On-treatment visits with history and physical exam should be $\leq$3 days before a scheduled dose of avelumab.
7. RC may be done any time after the pre-surgery evaluation is completed, but no later than day 64.
8. History and physical exams performed after RC (with the exception of the end of study visit) have a time window of +/- 7 days.
9. Post-operative labs up to day 60 after RC have a time window of +/- 7 days.

7.2 Study Procedures

7.2.1 Medical history and physical exam

The following needs to be documented:

- For enrollment history and physical exam:
  - complete history of present illness
  - past medical history
  - social history (including smoking history)
  - family history
  - allergy list
  - medication list including dosages and frequency of administration
  - full twelve point review of systems
  - vital signs
  - 10 system physical exam including ECOG PS
  - laboratory data and imaging
  - TURBT pathology specimen report
  - assessment/plan.
- For routine history and physical exam:
  - ECOG PS
  - presence or absence of adverse effects for visits after treatment has started (including management of such events)
  - full twelve point review of systems
  - vital signs
  - 10 system physical exam
  - list of medications including dosages and frequency of administration
  - dose and rate of avelumab being given
  - laboratory data and imaging
  - assessment and plan.
- End of treatment visit must include all the components of a routine history and physical.
- End of study visit must include all the components of a routine history and physical (minus dosage/rate of avelumab) and pathology results from RC.

7.2.2 Radical cystectomy

Radical cystectomy procedure is to be done per standard clinic practice.
In men this procedure involves removal of the bladder and prostate. In women it involves removal of the bladder and oftentimes a hysterectomy as well. In both sexes the procedure is followed by formation of a urinary diversion. The forms of urinary diversion include formation of an ileal conduit (where the ureters empty into the ileum) or formation of an internal reservoir, such as neobladder or continent cutaneous diversion. Relative contraindications to these internal drainage procedures include Tis in the prostatic ducts or a positive urethral margin. (63)

Bilateral pelvic lymph node dissection (LND) is considered standard of care and will be pursued in all patients with no set criteria for dissection template or number of LNs. Unusual circumstances, such as prior LND for another malignancy, significant scarring or inflammation, may preclude a full LND in some patients and will not qualify as a protocol deviation.

7.2.3 Radiographic imaging

- CT and/or MRI imaging will be used to determine suitability for study, adequately stage patients and determine presence or absence of progression.

7.2.4 Clinical laboratory tests

- CBC with diff – will be used to evaluate for autoimmune cytopenias.
- CMP – will be used to evaluate for electrolyte abnormalities, autoimmune nephritis or autoimmune hepatitis.
- UPT – will be performed as indicated in the study calendar for females of CBP.
- Free T4 – to evaluate for autoimmune hypothyroidism or hyperthyroidism.
- TSH – to evaluate for signs of hypophysitis, autoimmune hypothyroidism or hyperthyroidism
- ACTH – this may be used to evaluate for signs of hypophysitis and/or adrenal insufficiency.
- Hepatitis B surface antigen/hepatitis C antibody/hepatitis C RNA panel/HIV – patients with chronic viral infections must be excluded from the administration of immune checkpoint inhibitors (e.g., avelumab) due to lack of published safety data.
- Hemoglobin A1C – to evaluate for worsening of glycemic control, new onset autoimmune diabetes mellitus or worsening of preexisting type I diabetes mellitus.

7.2.5 Follow-up schedule

- Any patient receiving ≥ 1 dose of avelumab will be followed up to 90 days after RC (or end of last cycle of avelumab for those not receiving RC) to evaluate for safety. The indicated follow-up is outlined in the study calendar.
- Those patients evaluable for secondary endpoints will be followed up on study to day 90 after RC as outlined in the study calendar.
- After the safety monitoring period of 90 days post RC those patients who are evaluable for secondary endpoints will be followed off study for recurrence and survival for up to 2 years after RC. This may be done by physician visits/imaging performed approximately every 3 months for those undergoing surveillance at Baylor affiliates or by phone conversations approximately every 3 months for those undergoing surveillance at practices outside of Baylor affiliates.

7.3 Biological Sampling Procedures

Tumor and/or bladder tissue will be collected at two primary time points during the study: at time of TURBT and at time of RC. Peripheral blood will be collected at the time points and for the tests indicated in the study calendar.

7.3.1 Biomarker evaluation methods

Immunohistochemistry:

Concentrations of PD-L1 protein expression on tumor cells or other cell types (e.g. immune cells) will be measured by IHC analysis of FFPE tissues from the TURBT and RC specimens. PD-L1 expression will be assessed using one of the following anti-human-PD-L1 antibodies, depending on initial studies of the relative staining patterns on serial sections: SP142 (Spring Bioscience) (64), SP263 (Ventana Medical Systems), or E1L3N (Cell Signaling Technologies). (65) PD-L1 levels will be evaluated according to the percentage of positively stained cells based on a score ranging from 0 to 3: 0 = <1%; 1 = 1 to <5%; 2 = 5 to <10%; 3 = >10% of cells positive. (66)

FFPE tissue from TURBT and RC specimens will also be stained with primary antibodies for lymphocytic markers CD1d, CD3, CD4, CD5, CD8, CD19 and CD24. This will be

followed by staining with a secondary antibody for horseradish peroxidase and then DAB or blue chromogranin. The results of these lymphocyte IHC stains will be appraised using a semiquantitative scale scored according to the percentage of positively stained cells in tumor or stroma as follows: 0=none, 1 = up to 33% cells in tumor were positive, 2 = 33-66% positive cells and 3=more than 66% cells positive. (67) Tissue sections will be scored by at least two independent staff pathologists.

RNA Extraction from FFPE Tissue:

We have compared three different methodologies head-to-head for extraction of high quality RNA from FFPE tissues, namely ThermoFisher (PureLink™ FFPE RNA Isolation Kit), Clontech (NucleoSpin® totalRNA FFPE) and Covaris (truXTRAC FFPE RNA Kit). Covaris FFPE RNA extraction resulted in the highest quality RNA, sufficient for downstream qPCR and even RNA seq analysis. The main advantages of the Covaris protocol includes its efficiency to completely deparaffinize without using high-temperature enzymatic degradation (which also degrades RNA) and the overall shorter time required to complete the overall protocol. Thus we will extract RNA from FFPE tissue using the protocol for the Covaris truXTRAC FFPE RNA Kit.

qRT-PCR:

RNA will be reverse transcribed according to the manufacturer's instructions with Superscript III First-Strand Synthesis (Invitrogen) to form cDNA. (68) Tagman probes or specific primers will be designed to detect the following genes IFN-γ, TNF-α, TNF-β, T-box protein in B-cells (T-bet), IL-2, IL-10, IL-35, FOXP-3, CTLA-4, LAG-3, PD-L1, Granzyme-A and Perforin-1.

7.3.2 Withdrawal of informed consent for use of biological samples

If a subject withdraws consent to the use of biological samples, the samples will be disposed of and destroyed, and the action documented.

The Principal Investigator:

- Ensures that biological samples from that subject, if stored at the study site, are immediately identified, disposed of /destroyed, and the action documented
- Ensures the laboratory(ies) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed/destroyed, the action documented and the signed document returned to the study site
- Ensures that the subject is informed about the sample disposal.

8. DRUG INFORMATION

Pfizer, Inc. manufactures Avelumab. Specific storage, preparation, and other information is contained within the *Investigational Product Manual for Active and Placebo MSB0010718C, Avelumab (v.3).*

Drug supply will be ordered from Pfizer via the Drug Supply Form.

9. SAFETY ASSESSMENT

9.1 Definition of an adverse event

An adverse event **(AE)** is defined as any **untoward medical occurrence** in a patient or clinical investigation subject following use of avelumab. There does **NOT** need to be a causal relationship between the event and the Pfizer product.

9.2 Definition of serious adverse events

- Results in death;
- Is life-threatening (i.e., causes an immediate risk of death);
- Results in inpatient hospitalization or prolongation of existing hospitalization;
- Results in a persistent or significant disability/incapacity (i.e., substantial disruption in the ability to conduct normal life functions); or
- Considered to be an important medical event

Serious adverse events (SAEs) during pregnancy are defined as follows:

- Spontaneous abortion (includes miscarriage and missed abortion)
- Stillbirth
- Congenital anomaly (including in an aborted fetus, a stillborn infant, or neonate that dies shortly after birth)
- Neonatal death within 1 month of birth
- Infant death that is considered possibly related to intrauterine exposure to avelumab

9.3 Definition of adverse events of special interest

Adverse events of special interest (AESIs) are events specific to the further understanding of the safety profile of avelumab and require close monitoring and rapid communication by the investigator to the sponsor. Avelumab AESIs may be serious or non-serious.

AESIs include immune-mediated reactions such as enterocolitis, dermatitis, hepatotoxicity or hepatitis, endocrinopathy, neuropathy, nephritis and pneumonitis, as well as infusion related reactions, serious allergic reactions and anaphylaxis.

9.4 Infusion related reactions and serious allergic reactions

The frequency of high grade infusion reactions in clinical trials with ICIs is rare. (16, 22, 69-80) A high frequency of mild infusion reactions, was observed with anti-PD-L1 and anti-PD-1 therapy (81).

As with the administration of any foreign protein and/or other biologic agents, reactions following the infusion of MAbs can be caused by various mechanisms, including acute anaphylactic (IgE-mediated) and anaphylactoid reactions against the MAb, and serum sickness. Hypersensitivity reactions as well as infusion-related reactions have been reported with anti-PD-L1 and anti-PD-1 therapy (71).

Acute allergic reactions may occur, may be severe, and may result in death. Acute allergic reactions may include hypotension, dyspnea, cyanosis, respiratory failure, urticaria, pruritis, angioedema, hypotonia, arthralgia, bronchospasm, wheeze, cough, dizziness, fatigue, headache, hypertension, myalgia, vomiting and unresponsiveness. The incidence of high grade (grade ≥ 3) allergic reactions (e.g., anaphylaxis) have an incidence of ≤ 1%. (16, 22, 69-80). The

typical onset is within 30 minutes to two hours after the initiation of drug infusion, although symptoms may be delayed for up to 24 hours. The majority of reactions occur after the first or second exposure to the agent, but between 10 and 30 percent occur during subsequent treatments (82).

Guidelines for management of subjects with hypersensitivity (including anaphylactic reaction) and infusion-related reactions are outlined in Section 5.2 and Table 6 (section 5.6).

9.5 Select immune mediated adverse events

- Immune mediated pneumonitis
  - Signs and symptoms of pneumonitis include dyspnea (53%), new or worsening cough (35%), chest pain (7%), and radiographic changes. Up to 33% of patients may be asymptomatic at the onset of pneumonitis. Fatal cases of pneumonitis have occurred in less than 0.5% of cases in clinical trials of PD-1/PD-L1 inhibitor monotherapy. A case series from Memorial Sloan Kettering and the Melanoma Institute of Australia reported a pneumonitis incidence of 3% in patients treated with PD-1 or PD-L1 inhibitor monotherapy, with only 2% of cases being fatal. Most cases are grade 1 to 2 and improve with instituting the recommended therapies (88%). The median time to onset is approximately 2.8 months. (72, 83)
- Immune mediated colitis
  - Signs and symptoms include diarrhea (loose stools) or bowel movements more than usual, blood in stool or dark/tarry/sticky stool or severe abdominal pain/tenderness. (72)
- Immune mediated hepatitis
  - Signs and symptoms include transaminase elevations, elevations in total bilirubin, yellowing of the skin or the whites of the nails, severe nausea or vomiting, right sided abdominal pain, drowsiness, dark or tea colored urine, bleeding or bruising more easily than normal, and feeling less hungry than usual. (72)
- Immune mediated endocrinopathy
  - Signs and symptoms – headaches that will not go away or unusual headaches, extreme tiredness, weight gain and/or weight loss, dizziness or fainting, changes in mood or behavior (e.g., decreased sex drive, irritability or forgetfulness), hair loss, feeling cold, constipation, voice gets deeper, increased thirst, or change in frequency of urination. (72)
- Adrenal crisis
  - Signs and symptoms may include – severe dehydration, hypotension or shock out of proportion to current illness. (72)
- Immune mediated nephritis
  - Signs and symptoms – increase in serum creatinine, decrease in the amount of urine output, blood in the urine, swelling of the ankles, or loss of appetite. (72)
- Immune mediated rash
  - Signs and symptoms – rash, itchiness of the skin, skin blisters, and ulcers in the mouth or other mucous membranes. Signs and symptoms of dermatitis should be considered immune-mediated when on avelumab unless another etiology has been found. (72)
- Immune mediated encephalitis and other immune mediated neurological syndromes
  - Signs and symptoms – headache, fever, tiredness or weakness, confusion or memory problems, sleepiness, hallucinations, seizures or stiff neck. (72)

- Immune mediated myocarditis
    - Signs and symptoms include chest pain, dyspnea, fatigue, myositis with elevated creatinine kinase and cardiac conduction abnormalities. (84) Severe immune mediated myocarditis is a rare event with PD-1 pathway inhibitors, reported in less than 0.10% of treated patients. Review of a large safety database by Bristol Myers Squib that included 20,594 patients treated with nivolumab (a PD-1 inhibitory antibody) or nivolumab + ipilimumab (a CTLA4 inhibitory antibody) found a 0.09% incidence of severe drug related immune mediated myocarditis. This event rate was lower for nivolumab monotherapy at 0.06%. (85)

9.6 Incidence of select adverse events with immune checkpoint inhibitors

Table 10: Incidence of Adverse Events with Immune Checkpoint Inhibitors

Adverse events	CTLA-4 inhibitors	PD-1 or PD-L1 inhibitors
Any high grade (≥ 3) event	20-55%	10-43%
Anemia	8% grade 1-2 3% grade 3-4	0-8% grade 1-2 0-17% grade 3-4
Common adverse events (almost always grade 1-2) include: arthralgia, asthenia, back pain, constipation, decreased appetite, fatigue, fever, musculoskeletal pain, nausea and vomiting.	3-42%	0-34%
Endocrinopathies (e.g, disorders affecting the adrenal, gonadal, pancreas, pituitary or thyroid glands)	Unclear incidence due to variable reporting across trials	Unclear incidence due to variable reporting across trials
Infusion reactions	< 1% grade ≥ 3	< 1% grade ≥ 3
High grade diarrhea and colitis	5-17% (intestinal perforation had an incidence of 1% across studies and death was 0.8%)	0-5%
Myocarditis	N/A	0.06% grade ≥ 3
Nephritis	Rare	0-3% grade ≥ 3
Neutropenia	Rare	Rare
Pneumonitis (usually mild)	5% grade 1-2 0-3% grade ≥ 3	1-5% grade 1-2 0-3% grade ≥ 3
Rash (usually mild)	18-40% grade 1-2	0-26% grade 1-2
Severe dermatologic reactions (including bullous pemphigoid, full thickness ulceration, hemorrhagic or	2.5%	Rare

necrotic rash, Steven Johnson syndrome and toxic epidermal necrolysis)		
Other rare events (including duodenitis, gastritis, hematologic events, hypercalcemia, neurologic events, pancreatitis, pleural effusion, pneumonia, polymyalgia rheumatica, sarcoidosis, systemic inflammatory response syndrome, uveitis and vasculitis)	Rare	Rare
Thrombocytopenia	Rare	0-17% grade 1-2
Transaminitis (usually mild)	1-10% grade ≥ 3	0-5% grade ≥3

Grade 1 = mild, grade 2 = moderate, grade 3 = severe, grade 4 = life threatening and grade 5 = death. Rare adverse events are limited to case reports and are generally < 1% across studies. Neurologic adverse events include things such as (e.g., abducens or facial nerve palsy, autoimmune neuropathy, encephalitis, Guillain-Barre Syndrome, motor dysfunction, myasthenia gravis or transverse myelitis). (16, 22, 69-80, 84-85)

9.7 Attribution/recording of adverse events and serious adverse events

All adverse events, regardless of relatedness, will be reported on the appropriate case report forms. Adverse events will be described by nature of the event, the duration (start and stop dates and times), grade, treatment, relation to study drug, or if unrelated then the cause will be reported, the clinical course of the patient and the final outcome of the event.

The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.03 can be downloaded from the CTEP website.

Each event should be evaluated for its relatedness to participation in the research:

- Definite: The AE *is clearly related* to the study treatment.
- Probable: The AE *is likely related* to the study treatment.
- Possible: The AE *may be related* to the study treatment.
- Unlikely: The AE *is doubtfully related* to the study treatment.
- Unrelated: The AE *is clearly NOT related* to the study treatment.

9.8 Study recording period and follow-up for adverse events

AEs and SAEs will be recorded from the time of first treatment with Avelumab, throughout the treatment period and including the follow-up period (90 days after RC). During the course of the study all AEs and SAEs will be proactively followed up for each subject. Every effort will be made to obtain a resolution for all events, even if the events continue after discontinuation/study completion.

If a subject discontinues treatment for reasons other than disease progression, and therefore continues to have tumor assessments, drug or procedure-related SAEs must be captured until

the patient is considered to have confirmed PD and will have no further tumor assessments. The investigator is responsible for following all SAEs until resolution, until the subject returns to baseline status, or until the condition has stabilized with the expectation that it will remain chronic, even if this extends beyond study participation.

9.9 Follow-up of unresolved events

Any AEs that are unresolved at the subject's last visit in the study are followed up by the investigator for as long as medically indicated, but without further recording in the adverse event log. After 90 days post-RC, only subjects with ongoing investigational product-related SAEs will continue to be followed for safety.

Pfizer retains the right to request additional information for any subject with ongoing AE(s)/SAE(s) at the end of the study, if judged necessary.

9.10 Post-study events

After the subject has been permanently withdrawn from the study, there is no obligation for the investigator to actively report information on new AEs or SAEs occurring in former study subjects after the 90-day post-RC safety follow-up period for patients treated with avelumab. However, if the investigator learns of any SAEs, including death, at any time after the subject has been permanently withdrawn from study, and he/she considers there is a reasonable possibility that the event is related to study treatment, the investigator will notify Pfizer immediately.

9.11 Reporting of Adverse Events

The investigator primary responsibilities in the safety reporting are to identify and follow-up on SAEs experienced by participants in the study and to forward the information to the local regulatory authorities and Pfizer, as required by local regulations (for regulatory reporting) and IIR agreement (for reporting to Pfizer).

The following reportable events must be submitted to Pfizer within 24 hours of knowledge of the event (or immediately for death or life-threatening events) using the provided *Investigator-Initiated Research Serious Adverse Event Form (IIR SAE)* with *the Pfizer Reportable Events Fax Cover Sheet* with each SAE submission.

- Serious Adverse Events
- Exposure during Pregnancy or Breastfeeding (even if not associated with an adverse event)
- Occupational exposure (even if not associated with an adverse event)
- Potential drug-induced liver injury (Hy's Law cases): These events are considered important medical events and should be reported as SAEs.

Detailed guidance on the safety reporting is provided in the *Safety Reporting Reference Manual.*

Contact information for submission of reportable events to Pfizer:

- Fax: Pfizer U.S. Clinical Trial Department, Fax 1-866-997-8322.

  or

- E-mail: USA.AEReporting@pfizer.com, specifying:
  - PROTOCOL:
  - SUBJECT:
  - SITE/PI:
  - SAE/ONSET:

Serious adverse events are to be reported to the Baylor College of Medicine (BCM) Institutional Review Board (IRB) according to the board's reporting requirements and required time frame.

Any event that is reportable to the BCM IRB must also be reported to the Dan L Duncan Comprehensive Cancer Center (DLDCCC) Data Review Committee (DRC) via the Patient Safety Officer at dldcc-pso@bcm.edu.

9.12 Reporting of Deaths

All deaths that occur during the study, or within the protocol-defined 90-day post-RC safety follow-up period must be reported as follows:

- Death that is clearly the result of disease progression should be documented but should not be reported as an SAE.
- Where death is not due (or not clearly due) to progression of the disease under study, the AE causing the death must be reported as a SAE within 24 hours. The

report should contain a comment regarding the co-involvement of progression of disease, if appropriate, and should assign main and contributory causes of death.

- Deaths with an unknown cause should always be reported as a SAE.

Deaths that occur following the protocol-defined 90-day post-RC safety follow-up period will
be documented as events for survival analysis, but will not be reported as a SAE.

9.13 Overdose

An overdose is defined as a subject receiving a dose of avelumab in excess of that specified in the protocol.

Any overdose of a study subject with avelumab, with or without associated AEs/SAEs, is required to be reported within 24 hours of knowledge of the event to Pfizer using the contact for SAE reporting. If the overdose results in an AE, the AE must be recorded as an AE. Overdose does not automatically make an AE serious, but if the consequences of the overdose are serious, for example death or hospitalization, the event is serious and must be recorded and reported as a SAE. There is currently no specific treatment in the event of an overdose of avelumab. The investigator will use clinical judgment to treat any overdose of avelumab.

9.14 Pregnancy

Pregnancy itself, or pregnancy of a subject's partner, is not regarded as an AE unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of contraception. Elective abortions without complications should not be handled as AEs. The outcome of any conception occurring from the date of the first dose until 90 days after the last dose (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality) should be followed up/documented even if the subject was withdrawn from study.

Pregnancy in a female subject who has received investigational product is required to be reported ***within 24 hours of knowledge of the event*** to the sponsor and Pfizer Patient Safety or designee using the designated safety contact provided under the SAE reporting section.

Subjects who become pregnant during the study period must not receive additional doses of investigational product but will not be withdrawn from the study. The pregnancy will be followed for outcome of the mother and child (including any premature terminations) and should be reported to Pfizer Patient Safety or designee after outcome.

Males should refrain from fathering a child or donating sperm during study and for 90 days following the last dose of avelumab.

Should the investigator become aware of a pregnancy in the partner of a male study subject who has received investigational product this should be reported ***within 24 hours of knowledge of the event*** to Pfizer Patient Safety or designee using the safety contact provided under the SAE reporting section. The sponsor will endeavor to collect follow-up information on such pregnancies provided the partner of the study subject provides consent.

The full details of reporting for any pregnancy that occurs while exposed to avelumab is described in the Pfizer *Safety Reporting Reference Manuel*.

10. STATISTICAL METHODS

10.1 Study design and sample size

This is a single arm, non-randomized window of opportunity study to see the change in T cell subpopulations (CD8, CD4 and/or CD3) in tumor samples collected pre- and post-treatment with avelumab. Assuming that pre- and post-treatment values within patients are moderately correlated ($r \geq 0.5$), then 10 patients with paired pre- and post-treatment samples will provide ~80% power to detect a 1 standard deviation change in T cell populations (alpha=5% two-tailed).

10.2 Statistical analysis

To see the change in T cells pre- and post-treatment, paired t-test or nonparametric Wilcoxon signed rank test will be used after checking its normality.
Pathological response rate will be estimated along with 95% confidence interval. Two year disease free survival will be calculated using Kaplan-Meier survival curves with estimates of median and 95% confidence interval. Patients who are decided not to be evaluable for the primary outcome will be considered evaluable for the safety analysis and secondary outcomes, such patients will be summarized descriptively. The correlation of translational studies with response parameters will be summarized descriptively. Changes from pre- and post- treatment will be assessed using paired tests. SAS software will be used for all statistical analyses.

11. ETHICAL AND REGULATORY REQUIREMENTS

11.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with the International Conference on Harmonization (ICH) /Good Clinical Practice, and applicable regulatory requirements for subject data protection.

11.2 Ethics and regulatory review

Avelumab is an investigational drug not approved for clinical use in patients with bladder cancer. Our study will undergo review by the BCM Institutional Review Board (IRB). Investigational New Drug applications will also be filed with the FDA.

11.3 Informed consent

Informed consent shall be documented by the use of a written consent form approved by the IRB and the sponsor. This consent form shall be signed and dated by the subject or the subject's legally authorized representative at the time of consent. A copy shall be given to the person signing the form.

11.4 Changes to the protocol and informed consent form

Any changes to the protocol or informed consent form will be reviewed by the sponsor and the local IRB prior to implementation. Any changes to the informed consent form that impact currently enrolled subjects (e.g., changes in procedures, changes in the benefit/risk profile) will require that subjects are re-consented with the new consent form.

11.5 Audits and inspections

All records and documents pertaining to the study will be maintained in appropriate permanent files as per the ICH guidelines for Essential Documents for the Conduct of a Clinical Trial and 21

CFR 11, and will be available for inspection by the Sponsor, Sponsor designee, the FDA, or any other designated review body at any time.

12. STUDY MANAGEMENT

The principal investigator assumes all oversight and reporting of the study, including the training of study site personnel and monitoring of the study.

12.1 Training of study site personnel

The PI of this study will train the study coordinator in the procedures necessary to identify and accrue appropriate individuals to the study. The PI of the study will also train the staff scientist in the lab on appropriate tissue collection and storage procedures. The PI will train infusion center staff on appropriate administration of study drugs.

12.2 Monitoring of the study

Subjects will be monitored closely throughout the course of the study via regular visits with the study PI and direct access to the PI throughout the duration of the study. The PI is directly responsible for the study subjects, and the study staff will be instructed to contact the PI immediately with any concerns regarding patient safety or other matters.

12.3 Study timetable and end of study

The end of the study will be defined as the time point 90 days after RC. The recruitment period is estimated to take place over a period of approximately 12 months.

13. DATA MANAGEMENT

The database that will be utilized for data collection and storage will be OnCore. The PI and study staff will adhere to the reporting requirements of the data to the institutional IRB.

14. DATA AND SAFETY MONITORING AND QUALITY ASSURANCE

This study will be monitored regularly by the Data Review Committee (DRC) of the Dan L. Duncan Comprehensive Cancer Center, at a frequency of at least once per year, in accordance with the DLDCCC Data and Safety Monitoring Plan. The DRC will monitor the study for progress and enrollment, toxicities, adverse events, and soundness of data.

This study will be monitored by the DLDCCC Quality Assurance program for study conduct and quality of data.

15. REFERENCES

1. Fairey AS, Daneshmand S, Quinn D, et al. **Neoadjuvant chemotherapy with gemcitabine/cisplatin vs. methotrexate/vinblastine/doxorubicin/cisplatin for muscle-invasive urothelial carcinoma of the bladder: a retrospective analysis from the University of Southern California.** *Urol Oncol*. 2013 Nov;31(8):1737-43.
2. Galsky MD, Pal SK, Chowdhury S, et al. **Comparative effectiveness of gemcitabine plus cisplatin versus methotrexate, vinblastine, doxorubicin, plus cisplatin as neoadjuvant chemotherapy for muscle-invasive bladder cancer.** *Cancer* 2015 Aug 1;121(15):2586-93.
3. Grossman HB, Natale RB, Tangen CM, et al. **Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer**. *New Engl J Med*. 2003 Aug 28;349(9):859-66.
4. Hernchenhorn D, Dienstmann R, Peixoto FA, et al. **Phase II trial of neoadjuvant gemcitabine and cisplatin in patients with resectable bladder carcinoma**. *Int Braz J Urol*. 2007 Sep-Oct;33(5):630-8.
5. International Collaboration of Trialists. **Neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder cancer: a randomized controlled trial**. *Lancet*. 1999 Aug 14;354(9178):533-40.
6. International Collaboration of Trialists. **International phase III trial assessing neoadjuvant cisplatin, methotrexate, and vinblastine chemotherapy for muscle-invasive bladder cancer: long-term results of the BA06 30894 trial.** J Clin Oncol. 2011 Jun 1;29(16):2171-7.
7. Plimack ER, Hoffman-Centis JH, Viterbo R, et al. **Accelerated methotrexate, vinblastine, doxorubicin, and cisplatin is safe, effective, and efficient neoadjuvant treatment for muscle-invasive bladder cancer: results of a multi-center phase II study with molecular correlates of response and toxicity**. *J Clin Oncol*. 2014 Jun 20;32(18):1895-901.
8. Van de Putte EE, Mertens LS, Meijer RP, et al. **Neoadjuvant induction dose-dense MVAC for muscle-invasive bladder cancer: efficacy and safety compared with classic MVAC and gemcitabine/cisplatin.** *World J Urol*. 2016 Feb;34(2):157-62.
9. Winquist E, Kirchner TS, Segal R, Chin J, Lukka H, Genitoruinary Cancer Disease Site Group, Cancer Care Ontario Program in Evidence-based Care Practice Guidelines Initiative. **Neoadjuvant chemotherapy for transitional cell carcinoma of the bladder: a systematic review and meta-analysis**. *J Urol*. 2004 Feb;171(2 Pt 1): 561-9.
10. Yeshchina O, Badalato GM, Wosnitzer MS, et al. **Relative efficacy of perioperative gemcitabine and cisplatin versus methotrexate, vinblastine, adriamycin, and cisplatin in the management of locally advanced urothelial carcinoma of the bladder**. *Urology* 2012 Feb;79(2):384-90.
11. Yuh BE, Ruel N, Wilson TG, et al. **Pooled analysis of clinical outcomes with neoadjuvant cisplatin and gemcitabine chemotherapy for muscle invasive bladder cancer.** *J Urol*, 2013 May;189(5):1682-6.
12. Advanced Bladder Cancer (ABC) Meta-Analysis Collaboration. **Neoadjuvant chemotherapy in muscle invasive bladder cancer: update of a systemic review and meta-analysis of individual patient data advanced bladder cancer (ABC) meta-analysis collaboration**. *Eur Urol*. 2005 Aug;48(2):202-5.

13. Bladder Cancer Guidelines Version 1.2017. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Bladder Cancer V.1.2017. © National Comprehensive Cancer Network, Inc 2017. All rights reserved. Accessed [January 4, 2017]. To view the most recent and complete version of the guideline, go online to NCCN.org. NATIONAL COMPREHENSIVE CANCER NETWORK®, NCCN®, NCCN GUIDELINES®, and all other NCCN Content are trademarks owned by the National Comprehensive Cancer Network, Inc.
14. Bellmunt J, Ribas A, Eres N, et al. **Carboplatin-based versus cisplatin-based chemotherapy in the treatment of surgically incurable advanced bladder carcinoma**. *Cancer*. 1997 Nov 15;80(10):1966-72.
15. Pardoll DM. **The blockade of immune checkpoints in cancer immunotherapy**. Nat Rev *Cancer*. 2012 Mar 22;12(4):252-64.
16. Hodi FS, O'Day SL, McDermott DF, et al. **Improved survival with ipilimumab in patients with metastatic melanoma**. *N Engl J Med*. 2010 Aug 19;363(8):711-23.
17. Wolchok JD, Weber JS, Maio M, et al. **Four-year survival rates for patients with metastatic melanoma who received ipilimumab in phase II clinical trials**. *Ann Oncol.* 2013 Aug;24(8):2174-80.
18. Liu J, Hamrouni A, Wolowiec D, et al. **Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN-{gamma} and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway**. *Blood*. 2007 Jul 1;110(1):296-304.
19. Taube JM, Anders RA, Young GD, et al. **Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape**. *Sci Transl Med*. 2012 Mar 28;4(127):127ra37.
20. Daud AI, Wolchok JD, Robert C, et al. **Programmed Death-Ligand 1 Expression and Response to the Anti-Programmed Death 1 Antibody Pembrolizumab in Melanoma**. *J Clin Oncol.* 2016 Oct 31 [Epub ahead of print]
21. Reck M, Rodriguez-Abreu D, Robinson AG, et al. **Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer**. *N Engl J Med*. 2016 Oct 8 [Epub ahead of print]
22. Rosenberg JE, Hoffman-Centis J, Powles T, et al. **Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicenter, phase 2 trial**. *Lancet.* 2016 May 7;387(10031): 1909-1920.
23. Huang RY, Eppolito C, Lele S, Shrikant P, Matsuzaki J and Odunsi K. **LAG3 and PD1 co-inhibitory molecules collaborate to limit CD8+ T cell signaling and dampen antitumor immunity in a murine ovarian cancer model**. *Oncotarget*. 2015 Sep 29;6(29):27359-77.
24. Woo SR, Turnis ME, Goldberg MV, et al. **Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape**. *Cancer Res*. 2012 Feb 15;72(4):917-27.
25. Sweis RF, Spranger S, Bao R, Paner GP, Stadler WM, Steinberg G and Gajewski TF. **Molecular Drivers of the Non-T-cell-Inflammed Tumor Microenvironment in Urothelial Bladder Cancer**. *Cancer Immunol Res*. 2016 Jul;4(7):563-8.
26. Pignot G, Goux CLE, Sibony M, et al. **mRNA expression levels and prognostic value PD1/PDL1 and CTLA4 pathway genes in a large series of 155 bladder tumors.** *J Clin Oncol* 34, 2016 (suppl; abstr 4523).
27. Carthon BC, Wolchok JD, Yuan J, et al. **Preoperative CTLA-4 blockade: tolerability and immune monitoring in the setting of a presurgical clinical trial**. *Clin Cancer Res*. 2010 May 15;16(10):2861-71.

28. Apolo AB, Infante JR, Hamid O, et al. **Avelumab (MSB0010718C; anti-PD-L1) in patients with metastatic urothelial carcinoma from the JAVELIN solid tumor phase Ib trial: Analysis of safety, clinical activity, and PD-L1 expression**. *J Clin Oncol* 34, 2016 (suppl; abstr 4514).
29. Massard C, Gordon MS, Sharma S, et al. **Safety and Efficacy of Durvalumab (MEDI4736), an Anti-Programmed Cell Death Ligand-1 Immune Checkpoint Inhibitor, in patients with Advanced Urothelial Bladder Cancer**. *J Clin Oncol*. 2016 Sep 10;34(26):3119-25.
30. Plimack ER, Bellmunt J, Gupta S, et al. **Pembrolizumab (MK-3475) in advanced urothelial cancer: Updated results and biomarker analysis from KEYNOTE-012.** *J Clin Oncol* 33, 2015 (suppl; abstr 4502).
31. Powles T, O'Donnell PH, Massard C, et al. **Updated efficacy and tolerability of durvalumab in locally advanced or metastatic urothelial carcinoma**. *J Clin Oncol* 35, 2017 (suppl 6S;abstr 286).
32. Sharma P, Callahan MK, Bono P, et al. **Nivolumab monotherapy in recurrent metastatic urothelial carcinoma (CheckMate 032): a multicentre, open-label, two-stage, multi-arm, phase I/II trial**. *Lancet Oncol*. 2016 Oct 7 [Epub ahead of print]
33. Bellmunt J, deWit R, Vaughn DJ, et al. Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma. N Engl J Med. 2017 Feb 17 [Epub ahead of print]
34. Balar AV, Galsky MD, Rosenberg JE, et al. **Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial**. *Lancet*. 2017 Jan 7;389(10064):67-76.
35. www.ascopost.com/News/44121. Accessed 11/15/2016.
36. Oing C, Rink M, Oechsle K, et al. **Second Line Chemotherapy for Advanced and Metastatic Urothelial Carcinoma: Vinflunine and Beyond-A Comprehensive Review of the Current Literature**. *J Urol,* 2016; 195(2), 254-263.
37. Bellmunt J, Théodore C, Demkov T, et al. **Phase III trial of vinflunine plus best supportive care compared with best supportive care alone after a platinum-containing regimen in patients with advanced transitional cell carcinoma of the urothelial tract**. *J Clin Oncol,* 2003; 27(27):4454-4461.
38. Uhm JE, Lim HY, Kim WS, et al. **Paclitaxel with cisplatin as salvage treatment for patients with previously treated advanced transitional cell carcinoma of the urothelial tract**. *Neoplasia,* 2007; 9:18-22. Pardoll DM. **The blockade of immune checkpoints in cancer immunotherapy.** *Nat Rev Cancer*. 2012 Mar 22;12(4):252-64.
39. Zelenay S, van der Veen AG, Bottcher JP, et al. **Cyclooxygenase-Dependent Tumor Growth through the Evasion of Immunity.** *Cell*. 2015 Sep 10;162(6):1257-70.
40. Kurtova AV, Xiao J, Mo Q, et al. **Blocking PGE2-induced tumor repopulation abrogates bladder cancer chemoresistance**. *Nature*. 2015 Jan 8;517(7533):209-13.
41. Baratelli F, Lin Y, Zhu L, et al. **Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4+ T cells**. *J Immunol*. 2005. Aug 1;175(3):1483-90.
42. Hangai S, Ao T, Kimura Y, et al. **PGE2 induced in and released by dying cells functions as an inhibitory DAMP**. *Proc Natl Acad Sci USA*. 2016 Apr 5;113(14):3844-9.
43. Sinha P, Clements VK, Fulton AM and Ostrand-Rosenberg S. **Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells**. *Cancer Res*. 2007 May 1;67(9):4507-13.

44. Naing A, Papadopoulos KP, Autio KA, et al. **Safety, Antitumor Activity, and Immune Activation of Pegylated Recombinant Human Interleukin-10 (AM0010) in Patients with Advanced Solid Tumors**. *J Clin Oncol*. 2016 Aug 15 [Epub ahead of print].
45. Tchekmedyian N, Gray JE, Creelan BC, et al. **Propelling Immunotherapy Combinations Into the Clinic**. *Oncology (Williston Park)*. 2015 Dec;29(12).
46. Van Overmeire E, Stijlemans B, Heymann F, et al. **M-CSF and GM-CSF Receptor Signaling Differentially Regulates Monocyte Maturation Macrophage Polarization in the Tumor Microenvironment**. *Cancer Res*. 2016 Jan 1;76(1):35-42.
47. Zhang H, Ye YL, Li MX, et al. **CXCL2/MIF-CXCR2 signaling promotes the recruitment of myeloid-derived suppressor cells and is correlated with prognosis in bladder cancer**. *Oncogene*. 2016 Oct 10 [Epub ahead of print]
48. Boyerinas B, Jochems C, Fantini M, Heery CR, Gulley JL, Tsang KY and Schlom J. **Antibody-Dependent Cellular Cytotoxicity Activity of a Novel Anti-PD-L1 Antibody Avelumab (MSB0010718C) on Human Tumor Cells**. *Cancer Immunol Res*. 2015 Oct;3(10):1148-57.
49. Intlekofer AM and Thompson CB. **At the bench: preclinical rationale for CTLA-4 and PD-1 blockade as cancer immunotherapy.** *J Leukoc Biol*. 2013 Jul;94(1):25-39.
50. Liu K, Tan S, Chai y, et al. **Structural basis of anti-PD-L1 monoclonal antibody avelumab for tumor therapy**. *Cell Res*. 2016 Aug 30 [Epub ahead of print].
51. Fujii R, Friedman ER, Richards J, Tsang KY, Heery CR, Schlom J and Hodge JW. **Enhanced killing of chordoma cells by antibody-dependent cell-mediated cytotoxicity employing the novel anti-PD-L1 antibody avelumab**. *Oncotarget*. 2016 Jun 7;7(23):33498-511.
52. Grenga I, Donahue RN, Lepone LM, Richards J and Schlom J. **A fully human IgG1 anti-PD-L1 MAb in an in vitro assay enhances antigen-specific T-cell responses**. *Clin Transl Immunology*. 2016 May 20;5(5):e83.
53. Vandeveer AJ, Fallon JK, Tighe R, Sabzevari H, Schlom J and Greiner JW. **Systemic Immunotherapy of Non-Muscle Invasive Mouse Bladder Cancer with Avelumab, an Anti-PD-L1 Immune Checkpoint Inhibitor**. *Cancer Immunol Res.* 2016 May 4;(5):452-62.
54. Gulley JL, Rajan A, Spigel DR, et al. **3090 Avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with metastatic or recurrent non-small-cell lung cancer progressing after platinum-based chemotherapy: A phase Ib trial**. *Eur J Cancer*. 2015 Sep;51(3):S629.
55. Dirix LY, Takacs I, Nikolinakos P, et al. **Avelumab (MSB0010718C), an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: A phase Ib JAVELIN solid tumor trial**. *2015 San Antonio Breast Cancer Symposium*. Abstract S1-04. Presented December 9, 2015.
56. Kaufman HL, Russell J, Hamid O, et al. **Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial**. *Lancet Oncol*. 2016 Oct;17(10):1374-1385.
57. Disis ML, Patel MR, Pant S, et al. **Avelumab (MSB0010718C; anti-PD-L1) in patients with recurrent/refractory ovarian cancer form the JAVELIN Solid Tumor Phase Ib trial: safety and clinical activity**. *J Clin Oncol* 34, 2016 (suppl; abstr 5533).
58. Hassan R, Thomas A, Patel MR, et al. **Avelumab (MSB0010718C; anti-PD-L1) in patients with advanced unresectable mesothelioma from the JAVELIN solid tumor phase Ib trial: Safety, clinical activity and PD-L1 expression**. *J Clin Oncol* 34, 2016 (suppl; abstr 8503).
59. Chung HC, Arkenau HT, Wyrwicz L, et al. **Avelumab (MSB0010718C; anti-PD-L1) in patients with advanced gastric or gastroesophageal junction cancer from JAVELIN**

solid tumor phase Ib trial: Analysis of safety and clinical activity. *J Clin Oncol* 34, 2016 (suppl; abstr 4009).

60. Winquist E, Kirchner TS, Segal R, Chin J, Lukka H, Genitourinary Cancer Disease Site Group, Cancer Care Ontario Program in Evidence-based Care Practice Guidelines Initiative. **Neoadjuvant chemotherapy for transitional cell carcinoma of the bladder: a systematic review and meta-analysis**. *J Urol*. 2004 Feb;171(2 Pt 1): 561-9.
61. von der Maase H, Hansen SW, Roberts JT, et al. **Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: results of a large, randomized, multinational, multicenter, phase III study.** *J Clin Oncol*. 2000 Sep;18(17):3068-77.
62. Sternberg CN, de Mulder PH, Schornagel JH, et al. **Seven year update of an EORTC phase III trial of high-dose intensity M-VAC chemotherapy and G-CSF versus classic M-VAC in advanced urothelial tract tumors.** *Eur J Cancer.* 2006 Jan;41(1):50-54.
63. **Bladder Cancer Guidelines Version 1.2017**. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Bladder Cancer V.1.2017. © National Comprehensive Cancer Network, Inc 2016. All rights reserved. Accessed [January 8, 2017]. To view the most recent and complete version of the guideline, go online to NCCN.org. NATIONAL COMPREHENSIVE CANCER NETWORK®, NCCN®, NCCN GUIDELINES®, and all other NCCN Content are trademarks owned by the National Comprehensive Cancer Network, Inc.
64. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. **Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients**. *Nature*. 2014;515:563-7.
65. Mahoney KM, Sun H, Liao X, Hua P, Callea M, Greenfield EA, et al. **PD-L1 Antibodies to Its Cytoplasmic Domain Most Clearly Delineate Cell Membranes in Immunohistochemical Staining of Tumor Cells**. *Cancer Immunol Res*. 2015;3:1308-15.
66. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, et al. **MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer**. *Nature*. 2014;515:558-62.
67. Matkowski R, Gisterek I, Halon A, et al. **The prognostic role of tumor-infiltrating CD4 and CD8 T lymphocytes in breast cancer**. *Anticancer Res*. 2009 Jul;29(7):2445-51.
68. Kurtova AV, Xiao J, Mo Q, et al. **Blocking PGE2-induced tumor repopulation abrogates bladder cancer chemoresistance**. *Nature*. 2015 Jan 8;517(7533):209-13.
69. Ansell SM, Lesokhin AM, Borrello I, et al. **PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma**. *N Engl J Med*. 2015 Jan 22;372(4):311-9.
70. Borghaei H, Paz-Ares L, Horn L, et al. **Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer**. *N Engl J Med*. 2015 Oct 22;373(17):1627-39.
71. Brahmer J, Reckamp KL, Baas P, et al. **Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer**. *N Engl J Med*. 2015 Jul 9;373(2):123-35.
72. Bristol-Myers Squibb. **Opdivo: Immune-Mediated Adverse Reactions Management Guide.** Pages 1-59. Accessed online on 5/31/2016.
73. Eggermont AM, Chiarion-Sileni V, Grob JJ, et al. **Adjuvant ipilimumab versus placebo after complete resection of high-risk stage III melanoma (EORC 18071): a randomized, double-blind, phase 3 trial**. *Lancet Oncol*. 2015 May;16(5):522-30.
74. Garon EB, Rizvi NA, Hui R, et al. **Pembrolizumab for the treatment of non-small-cell lung cancer**. *N Engl J Med*. 2015 May 21;372(21):2018-28.
75. Le DT, Uram JM, Wang H, et al. **PD-1 Blockade in Tumors with Mismatch-Repair Deficiency**. *N Engl J Med*. 2015 Jun 25;372(26):2509-20.

76. Motzer RJ, Escudier B, McDermott DF, et al. **Nivolumab versus Everolimus in Advanced Renal Cell Carcinoma**. *N Engl J Med*. 2015 Nov 5;373(19):1803-13.
77. Nanda R, Chow LQ, Dees EC, et al. **Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study**. *J Clin Oncol*. 2016 May 2 [Epub Ahead of Print]
78. Postow MA, Callahan MK and Wolchok JD. **Immune Checkpoint Blockade in Cancer Therapy.** *J Clin Oncol*. 2015 Jun 10;33(17):1974-82.
79. Robert C, Long GV, Brady V, et al. **Nivolumab in previously untreated melanoma without BRAF mutation**. *N Engl J Med*. 2015 Jan 22;372(4):320-30.
80. Robert C, Schachter J, Long GV, et al. **Pembrolizumab versus Ipilimumab in Advanced Melanoma**. *N Engl J Med*. 2015 Jun 25;372(26):2521-32.
81. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. **Safety and activity of anti-PD-L1 antibody in patients with advanced cancer**. *N Engl J Med*. 2012;366(26):2455- 65.
82. Lenz HJ. **Management and preparedness for infusion and hypersensitivity reactions**. *Oncologist*. 2007;12(5):601-9.
83. Naidoo J, Wang X, Woo KM, et al. **Pneumonitis in Patients Treated with Anti-Programmed Death-1/Programmed Death Ligand 1 Therapy**. *J Clin Oncol*. 2016 Sep 19 [Epub ahead of print]
84. Johnson DB, Balko JM, Compton ML, et al. **Fulminant Myocarditis with Combination Immune Checkpoint Blockade**. *N Engl J Med*. 2016 Nov 3;375(18):1749-55.
85. http://www.ascopost.com/issues/december-10-2016/cardiac-complications-in-patients-receiving-combination-checkpoint-inhibitors-are-rare-but-can-be-fatal/. Accessed on 12/12/2016.