Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features

Thesis

Submitted to

All India Institute of Medical Sciences, Jodhpur

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Master of Chirurgiae (MCh)

Urology

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DECLARATION

I hereby declare that this thesis titled **"Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features"** is a bonafide and original research work carried out in partial fulfilment of the requirements for the degree of Master of Chirurgiae (M.Ch.) in Urology under supervision and guidance, in the Department of Urology, All India Institute of Medical Sciences, Jodhpur.

I further state that no part of the thesis has been submitted either in part or in full for any other degree of All India Institute of Medical Sciences or any other institute/university.

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CERTIFICATE

This is to certify that the thesis titled “Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features” is the bonafide work of Dr. Amit Aggarwal carried out under our guidance and supervision, in the Department of Urology, All India Institute of Medical Sciences, Jodhpur.

It is further certified that the candidate has fulfilled the pre-requisites necessary for the submission of this thesis work.

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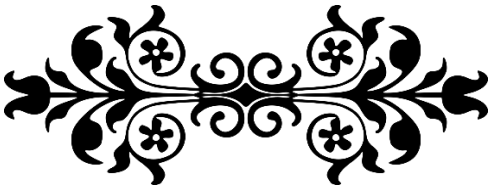


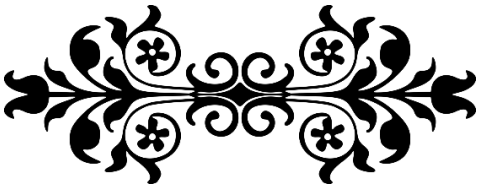
DEDICATED

TO

MY FAMILY

&

TEACHERS



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helped immensely in wrapping up this study successfully. I humbly thank God for his blessings and bow down in hope of continued grace in this endeavor of becoming a compassionate doctor.

INDEX

LIST OF ABBREVIATIONS

AJCC- American Joint Committee on Cancer

APC- Antigen presenting cell

AR- androgen receptor

AREs- Androgen Response Elements

AUA- American Urological Association

BCG- Bacillus Calmette Guérin

Bcl-2- B cell lymphoma 2

Ca- Carcinoma

cc- clear cell

CPI- Immune Checkpoint Inhibitors

CRPC- Castrate resistant prostate carcinoma

CT- Computed Tomography

CTL- Cytotoxic T lymphocyte

CTLA- Cytotoxic T lymphocyte associated protein

DHT- Dihydrotestosterone

EBRT- External Beam Radiotherapy

ECM- Extracellular matrix

ECOG- Eastern cooperative oncology group

ER alpha- estrogen receptor alpha

ER beta- estrogen receptor beta

FasL- Fas ligand

FDA- Food and Drug Administration

FGFR- Fibroblast growth factor receptor

GnRH- Gonadotropin Releasing Hormone

GSTM1-Glutathione S-Transferase

GU- Genitourinary

HIF-1α- Hypoxia-inducible factor alpha

HLA- Human leukocyte antigen

HSPs- Heat Shock Proteins

IFN-γ- Interferon gamma

IgG- Immunoglobulin G

IHC- Immunohistochemistry

LBD- Ligand Binding Domain

MHC- Major histocompatibility complex

MRI- Magnetic Resonance Imaging

mRNA- messenger ribonucleic acid

NAT2- N-acetyltransferase 2

NCCN- National Comprehensive Cancer Network

NMIBC- Non Muscle Invasive Bladder Carcinoma

NTD- N-terminal Domain

OS- Overall survival

PCa- Prostate cancer

PD-1- Programmed Cell Death-1

PD-L1- Programmed Cell Death Ligand-1

PI3K- phosphoinositide 3-kinase

PSA- Prostate specific antigen

RCC- Renal cell carcinoma

RR- Relative Risk

TC- Tumor cell

TII- Timor infiltrating immune cells

TILs- Tumor infiltrating lymphocytes

TLR- Toll like receptor

TNM- Tumor, Nodes, and Metastases

UC- Urothelial carcinoma

VEGF- Vascular endothelial growth factor

WHO- World Health Organization

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SUMMARY

STUDY DESIGN: Prospective Observational Study

OBJECTIVE: To examine Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features

BACKGROUND: The genitourinary (GU) malignancies constitute a heterogeneous group of diseases affecting the kidney, renal collecting system, bladder, prostate, testes, and penis, with each malignancy having a distinct biology and clinical outcomes. The advent of more sophisticated immunotherapies in the form of immune checkpoint inhibitors (CPIs)—monoclonal antibodies targeting specific regulatory immune factors—has dramatically changed the landscape of cancer treatment. Our study is to assess the clinicopathological impact of the expression of PD-L1 in genitourinary malignancy in a tertiary care centre in Rajasthan, India.

METHODS: This study was conducted from December 2020 to November 2022 at the Department of Urology and Department of Pathology, All India Institute Of Medical Sciences, Jodhpur, Rajasthan, India. All patients diagnosed with genitourinary mass and undergoing surgery or biopsy for the same and diagnosed with malignancy on histopathological examination (HPE) were included. Patients with benign pathology on HPE, not willing to participate in the study and patients with penile malignancy were excluded.

RESULTS: A total of 97 patients were included in the study. 38(39.2%) patients were of urothelial carcinoma, 30(30.9%) patients were of renal cell carcinoma and 29(29.9%) patients were of prostate carcinoma. Out of these, 41.4% of prostate carcinoma, 71.1% of urothelial carcinoma and 60% of renal cell carcinoma patients were positive for PD-L1 expression. There were many differences in PD-L1 expression amongst various studied clinicopathological factors but statistical significant difference was seen in high and low grade urothelial carcinoma.

CONCLUSION: Our study is the one with the large sample size conducted on the Indian population of various genitourinary malignancies. There were many differences among PD-L1 expression in GU malignancies but in urothelial carcinoma, high grade tumors had statistically significant higher PD-L1 expression as compared to low grade tumors. Such great variation shows that further studies are required to answer the question of judicious

implication of therapeutic agents targeting these receptors for benefitting patients who do not respond to or progress on conventional therapeutic modalities.


INTRODUCTION

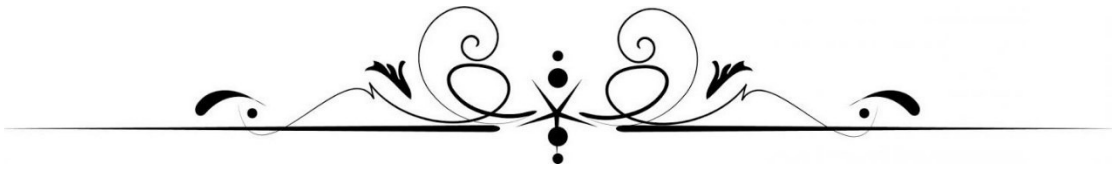
INTRODUCTION

The genitourinary (GU) malignancies constitute a heterogeneous group of diseases affecting the kidney, renal collecting system, bladder, prostate, testes, and penis, with each malignancy having a distinct biology and clinical outcomes. Treatment of these malignancies therefore involves unique approaches with respect to the roles of surgery, radiation, and systemic therapy. Almost all modalities of systemic treatment have been used in the management of metastatic GU cancers, including cytotoxic chemotherapy, antiangiogenic therapies, and hormonal treatments. Immune-based treatments have also previously been used with some benefit in GU malignancies—for example, cytokine treatments for advanced renal cell carcinoma (RCC) and intravesical instillation of Bacillus Calmette–Guérin (BCG) for treatment of non-muscle-invasive bladder cancer (NMIBC).

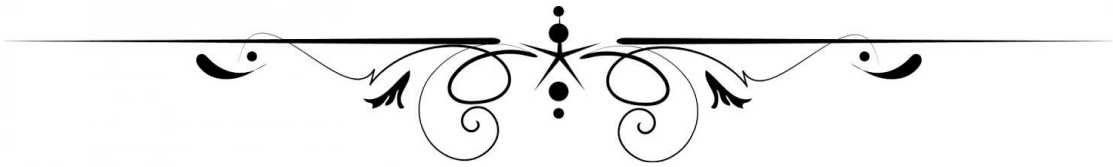
The advent of more sophisticated immunotherapies in the form of immune checkpoint inhibitors (CPIs)—monoclonal antibodies targeting specific regulatory immune factors—has dramatically changed the landscape of cancer treatment. The most prominent of the monoclonal antibodies currently in use target the Cytotoxic T Lymphocyte Associated Protein- 4 (CTLA-4) and Programmed Death-1(PD-1) or PD-L1 pathways. Those therapies have been evaluated in numerous clinical trials in GU oncology, with new data changing the treatment of GU malignancies at a rapid pace.

PD-1 on T cells interact with its ligand PD-L1 on tumor cell and leads to inhibited function of effector T cells; therefore, tumors escape from T-cell regulated immune response by utilizing the PD-1/PD-L1 signaling pathway. PD-1/PD-L1 inhibitors have shown survival benefits in various advanced cancers, including melanoma, lymphoma, renal cell carcinoma, and urothelial carcinoma (UC). PD-L1 status has been demonstrated to significantly correlate with response and survival improvement from anti-PD-1/PD-L1 immunotherapy in UC patients, while there is no convincing evidence whether PD-L1 expression in tumor cells (TCs) or tumor infiltrating immune cells (TIICs) with a cut-off value of 5% or 1% could predict the prognosis and response.

Our study is to assess the clinicopathological impact of the expression of PD-L1 in genitourinary malignancy in a tertiary care centre in Rajasthan, India.



REVIEW OF LITERATURE



REVIEW OF LITERATURE

For most advanced malignancy, chemotherapy is the primary modality of treatment. Although chemotherapy remains the treatment of choice for most advanced cancers, harnessing the power of the host's immune system to identify and regain control of cancerous cells has been an area of therapeutic research. The ability of tumour cells to avoid immune destruction (immune escape) is another key barrier to the successful management of cancer. An important mechanism of cancer immune escape involves binding of the cell surface receptor Programmed Death 1 (PD-1) on cytotoxic T lymphocytes (CTLs) with cell surface Programmed Death Ligand 1 (PD-L1) on cancerous cells(1). The PD-1/PD-L1 axis is one of many "immune checkpoint regulators" hardwired into our immune system to maintain self-tolerance and limit the duration and amplitude of the immune response to prevent collateral tissue damage(2). Tumour cells take advantage of this endogenous mechanism of immune suppression, and activation of the PD-1/PD-L1 axis results in suppression of anti-tumour adaptive immunity through mechanisms involving induction of CTL anergy, exhaustion and apoptosis(1,3). In addition to interfering with CTL function, engagement of PD-1 with PD-L1 increases tumour cell resistance to pro-apoptotic signals such as those delivered by cytotoxic immune effectors(4). PD-L1 has emerged as a valuable prognostic marker and several studies have correlated PD-L1 expression with tumour infiltrating lymphocytes (TILs)(5–7), high histological grade(8) and a negative prognostic factor for overall survival(9). There exists a strong correlation between tumour PD-L1 expression and response to anti-PD-1 treatment(10,11). Based on the knowledge that PD-L1 expression could protect tumour cells from triggers of apoptosis(4) and that the PD-1/PD-L1 axis may be correlated with negative patient outcomes(9).

The Immune System

The key function of the immune system is to continuously scan and defend the body from intruder pathogens and cancerous cells(12). The immune system is composed of diverse, yet very specialized cells that work in an orchestrated way to initiate an immune response against any pathogenic invaders and also against cells showing signs of becoming malignant(13). The immune system comprises of primarily two branches: innate and adaptive immunity(14). The innate immune system is the first line of defense, however, it lacks the specificity to identify individual pathogens and does not develop any memory against these specific pathogens(15). The recognition of intruders via the innate system is general and depends on

innate receptors like Toll-Like Receptors (TLRs) family(15). TLRs mediate recognition of pathogens membrane signature molecules such as proteins, lipoproteins, and lipid(15). The innate immune system also alarms the adaptive immune system for the presence of harmful molecules(16). Specialized Antigen Presenting Cells (APCs) of the innate immune system shuttle parts of the pathogens and present them to the adaptive immune cells, triggering the development of a more selective and powerful adaptive immune system(17). Along with cell-mediated responses, adaptive immunity also comprises of a humoral response(14). T lymphocytes play a major role in activating humoral responses, T cells are specialized in distinguishing and initiating an immune response against non-self antigens, at the same time, T cells maintain tolerance while distinguishing self-cell antigens(18).

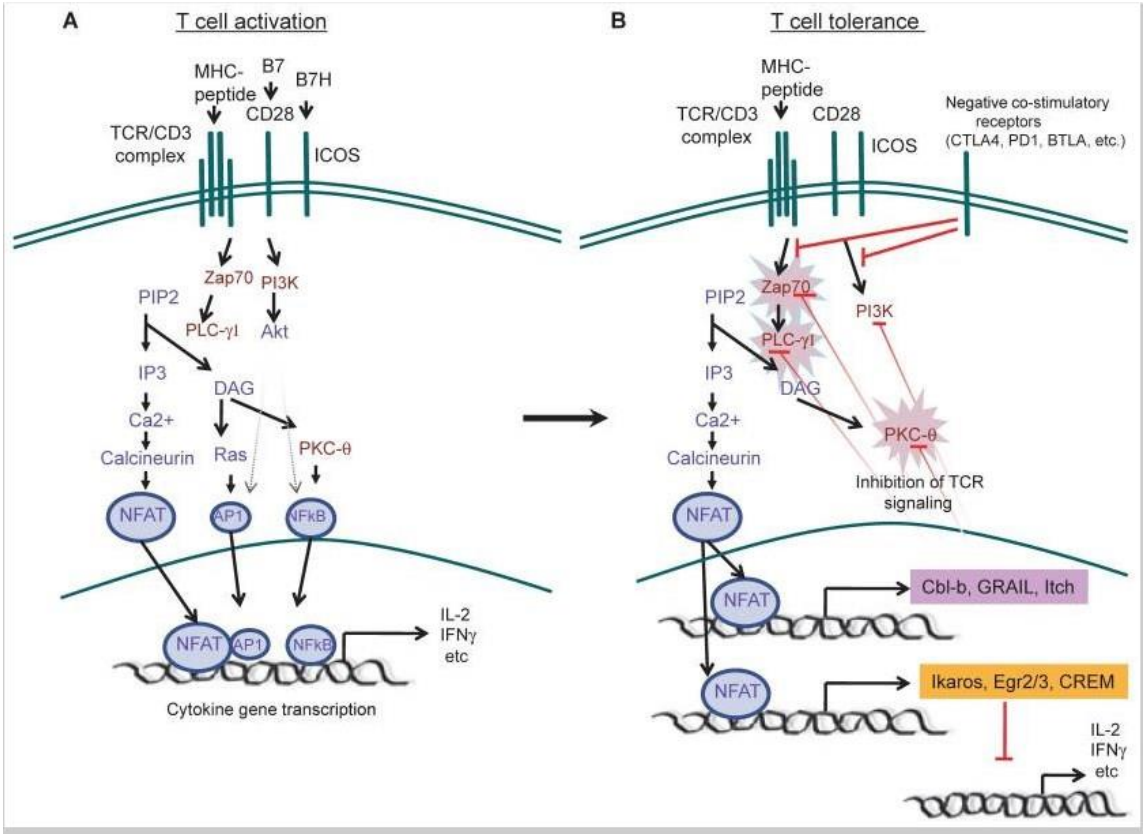


Figure 1: Molecular mechanisms of T cell tolerance.(19)

Many of these negative and positive co-stimulatory molecules have been revealed. Examples of positive regulatory molecules are CD28, inducible co-stimulator (ICOS) and T cell suppressing molecules include cytotoxic T-lymphocyte antigen-4 (CTLA4), PD-1, B7S1, and B7-H3(19). A review by Nurievav et al, 2016 highlighted various molecules determining the fate of the precursor T cells in the periphery, mechanisms, and the global intra-cellular

pathways leading to T cell activation (proliferation and expansion), or T cell silencing (suppression)(19). Panel A of Figure 1 illustrates how TCR establishes the first recognition to MHC carrying a peptide, followed by a second signaling molecule (positive co-stimulatory molecules like B7 (CD28) and B7H (ICOS) which alters global downstream pathways like nuclear factor of activated T cells (NFAT), activating protein- 1 (AP-1), and NF-κB, resulting in changes important pro-inflammatory cytokine production and release like IL-2 and IFN gamma important for initiating an immune response(19). Panel 2 of Figure 1 shows the immune silencing of T cells that can be dictated by the presentation of a negative co-stimulatory (inhibitory) molecules such as CTLA4, PD-1, and BTLA.

PD-1/PD-L1

The Programmed Death 1/Programmed Death Ligand 1 (PD-1/PD-L1) axis functions to dampen or suppress the adaptive immune system through inhibition of the T cell response. Physiologically, PD-1/PD-L1 engagement serves to induce self-tolerance and control the amplitude of an immune response to prevent tissue damage(2,20–22). It also represents an important immunological synapse in the context of cancer immune evasion.

Programmed Death 1 (PD-1) is a type-1 transmembrane protein of the CD28 family of molecules and is found on activated $CD4^+$, $CD8^+$, regulatory and NK T cells, and on B-cells(23–26). PD-1 is not detected on resting T cells but is induced upon activation and interaction with T-cell receptor (TCR)/B-cell receptor (BCR)(20). The important immunological role of PD-1 was revealed shortly after its discovery when PD-1 knockout mice showed a propensity towards an autoimmune phenotype(20,21). PD-1 engagement with one of its two natural ligands, Programmed Death Ligand-1 (PD-L1)(27–29) and Programmed Death Ligand-2 (PD-L2)(29), results in negative T cell activation and regulation. Although the interaction of PD-1 with both PD-L1 and PD-L2 results in T cell suppression, only PD-L1 is expressed in a majority of solid tumours; this makes PD-L1 the more relevant of the two ligands in the context of cancer and immunotherapy (discussed below).

PD-L1 (B7-H1, CD274) was the third member of the B7 family of molecules to be identified and found to have an inhibitory effect on T cell function upon engagement with its receptor, PD-1(27,28). The CD274 gene on human chromosome 9 encodes PD-L1. It is a type-1 protein consisting of a hydrophobic transmembrane domain and a cytoplasmic tail. It is

composed of 290 amino acids with immunoglobulin V-like and C-like domains, and is thus considered to be a member of the immunoglobulin superfamily(27).

When PD-L1 binds to PD-1, T cell function is inhibited or impaired through the phosphorylation and resultant deactivation of downstream signal transducers involved in T cell activation and proliferation, specifically those in the PI3K/AKT pathway, resulting in decreased cytokine expression (IFN- γ, TNF-α), decreased expression of survival factor Bcl-xl and decreased expression of key transcription factors involved in $CD8^+$ cytotoxic effector function(28,29).

Studies investigating PD-1 expression on tumour infiltrating lymphocytes (TILs) have shown that $CD8^+$ TILs express higher levels of PD-1 than their normal tissue or peripheral blood lymphocyte counterparts(30,31), thus highlighting the importance of this immune checkpoint mechanism in cancer immune evasion. It has been proposed that the PD-1/PD-L1 axis participates in tumour-cell immune evasion via induction of CTL apoptosis(32), anergy (lack of responsiveness to antigen)(33), and exhaustion(34), as well as Treg cell-mediated suppression (Figure 2).

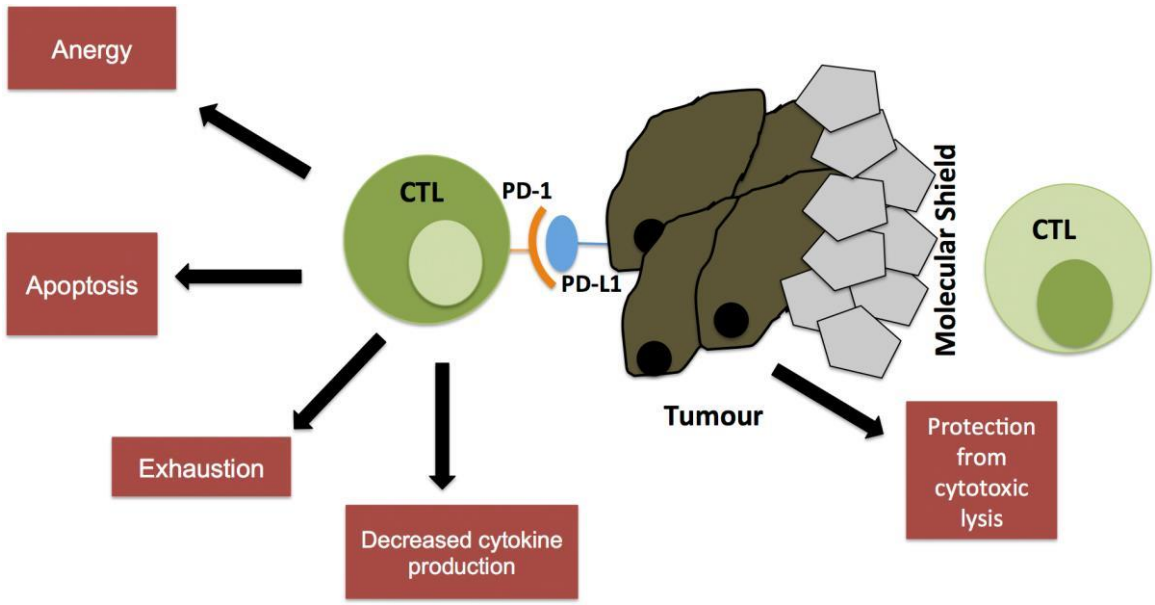


Figure 2: PD-1/PD-L1-mediated mechanisms of tumour immune escape (3)

The interaction between PD-1 on CTLs and PD-L1 on tumour cells results in T cell dysfunction via induction of anergy, apoptosis, exhaustion and decreased cytokine secretion. Furthermore, PD-1/PD-L1 promotes tumour cell resistance to CTL-mediated lysis. Figure is adapted from Zou et al.[3].

Although the presence of PD-L1 on most solid tumour cells is well established, levels of PD-L1 expression may be associated with different molecular cancer types and various pathological/clinical associations(3,11)

With regard to prostate cancer (PCa), many Pca specimens have been shown to express relatively low levels of PD-L1 compared to other solid tumours; however,TILs from prostate cancer patients express high levels of PD-1(31) and the levels of this molecule are increased on T cells surrounding the cancerous lesions(35). Recently a study found increased levels of PD-L1 in enzalutamide-resistant prostate cancer(36), implicating the PD- 1/PD-L1 axis in a more aggressive, treatment-resistant phenotype.

Journey of Immune checkpoint inhibitors in prostate carcinoma

Prostate cancer is the most common malignant neoplasm in men, other than non-melanoma skin cancer(37). Although a great proportion of patients may be cured with local therapies, a significant fraction will develop recurrent and metastatic disease.

Sipuleucel-T, an autologous active cellular immunotherapy, was approved by the FDA in 2010 for asymptomatic or minimally symptomatic patients with mCRPC, resulting in a 4.1-month improvement in median OS(38). Despite the relatively modest clinical benefit, the development of sipuleucel-T provided the foundation to further investigate immunotherapy in prostate cancer. Since the emergence of sipuleucel-T, there have been few successes and many failures in terms of further improving OS in men with advanced disease using the immune system to eliminate cancer. For example, cell-based and viral-based vaccines have shown largely disappointing results in patients with mCRPC(39).

In 2018, Drs. James Allison and Tasuku Honjo were awarded the Noble Prize in Physiology or Medicine for their research on immune checkpoints and uncovering ways to activate the immune system to attack cancer. These breakthrough discoveries have resulted in the development of several clinical immune checkpoint inhibitors that are changing the natural history of various malignancies including melanoma, lung cancer, renal cell carcinoma,

urothelial carcinoma, among others(40). Since immune checkpoint blockade has emerged as a promising treatment strategy for several tumor types, it has also been tested recently in patients with prostate cancer.

The first immune checkpoint to be studied in prostate cancer, thanks primarily to Dr. Allison, was cytotoxic T lymphocyte-associated protein-4 (CTLA-4) which led to the development of a fully human monoclonal antibody blocking the CTLA-4 pathway, called ipilimumab(41). As a result, two large phase III clinical trials were conducted to investigate the role of ipilimumab in mCRPC (pre- and post-docetaxel chemotherapy)(42,43). Unfortunately, these trials did not meet their primary endpoint of improving OS compared to placebo. Efforts to develop biomarkers to select specific prostate cancer patients who may benefit from this treatment strategy are needed.

Dr. Honjo's research has investigated the role of the programmed death-1 (PD-1) T-cell receptor and its ligand PD-L1 in maintaining an immunosuppressive tumor microenvironment(2). Overcoming this adaptive mechanism of immune escape using agents inhibiting the PD-1/PD-L1 axis may result in effective T-cell responses against cancer cells(44). However, in the first phase I study to investigate the role of a PD-1 inhibitor (nivolumab) in multiple malignancies, no encouraging responses were observed in seven prostate cancer patients enrolled in that study(45).

A phase II trial evaluated the role of a different PD-1 blocker, pembrolizumab, in prostate cancer patients who had failed enzalutamide. Four of 20 patients treated with pembrolizumab plus ongoing enzalutamide therapy had significant radiographic or prostate-specific antigen (PSA) responses. Interestingly, biomarker analysis revealed that one responder had DNA mismatch-repair deficiency and microsatellite instability(46), supporting the hypothesis that a high mutational (and neoantigen) load may be associated with better responses to immune checkpoint inhibition(47).

More recently, results from the KEYNOTE-028 trial of pembrolizumab in advanced cancers were published, suggesting antitumor activity in a subset of patients with prostate cancer. In this multicenter open-label basket trial, the efficacy and safety of pembrolizumab in patients with PD-L1-positive advanced cancers was investigated. In the prostate cancer cohort, 245 mCRPC patients were screened for PD-L1 expression in tumor cells or immune cells, and 35 men (14%) were considered PD-L1-positive, forming the evaluable study population. It is

important to note that previous pathology studies exploring PD-L1 expression in prostate cancer have reported variable frequencies of PD-L1 positivity. The largest study evaluated PD-L1 expression in 539 primary prostate cancer specimens and 57 cases of mCRPC. That study showed that PD-L1 expression in primary prostate cancers was observed in 8% of cases, while 32% of mCRPC samples were considered PD-L1-positive(48), suggesting that advanced castrate-resistant prostate cancer (CRPC) clones may use this pathway to escape immune system surveillance.

Returning to the results of the prostate cancer cohort of the KEYNOTE-028 study, 23 out of the 35 PD-L1- positive patients received pembrolizumab at a dose of 10 mg/kg intravenously every 2 weeks, for 24 months or until disease progression or unacceptable adverse events. The overall response rate (ORR) was 17% (4 patients), and no complete responses (CR) were identified. Stable disease (SD) was observed in 8 patients (35%). One patient had an unconfirmed partial response (PR), which may increase the ORR to 22%. Median duration of benefit (among responding patients) was 13 months. Importantly, progressive disease (PD) was the best response in 9 patients (39%), suggesting that many prostate cancer patients do no derive any benefit from pembrolizumab. Median OS for the entire prostate cancer population was 8 months, which is in the expected range of OS for this patient population.
Even more recently, at the 2018 American Society of Clinical Oncology (ASCO) Annual Meeting, results from the KEYNOTE-199 study were presented. This large phase II study evaluated the role of pembrolizumab in 258 patients with mCRPC following docetaxel treatment. Patients were enrolled into 3 cohorts based on disease and PD-L1 characteristics (cohort 1: soft-tissue disease and PD-L1-positive; cohort 2: soft-tissue disease and PD-L1-negative; cohort 3: bone-predominant disease irrespective of PD-L1 status) to evaluate antitumor activity with this PD-1 inhibitor. Patients enrolled in all cohorts received pembrolizumab 200 mg intravenously every 3 weeks, for 35 cycles or until disease progression or unacceptable toxicity(49). In this trial, pembrolizumab showed equivalent activity in PD-L1-positive and PD-L1-negative soft-tissue cohorts, and also looked promising in patients with bone-predominant disease. Although the ORR was only approximately 4%, about 9% of patients had durable response or SD (lasting >6 months). Importantly, 2 patients achieved a CR in the PD-L1-positive cohort. At the time of cut off analysis, approximately 10% of patients were still on treatment and the main cause of treatment discontinuation was PD. No treatment-related discontinuations or deaths were observed, and the safety profile of pembrolizumab was consistent with previous use of this agent in other tumor types.

Therefore, the combined results of the KEYNOTE-028 and -199 studies suggest that a small but meaningful proportion of mCRPC patients do benefit from single-agent PD-1 inhibitor treatment, and that these antitumor responses may be very durable in some patients.

Journey of Immune checkpoint inhibitors in renal cell carcinoma

Renal cell carcinoma (RCC) accounts for about 2% of cancer diagnosis and deaths globally(50). Renal cell tumors represent a group of histologically and molecularly heterogeneous diseases. The histologic classification of RCC has significantly changed in the last few decades, however several new entities were added based on either pathologic features or distinctive molecular alterations(51). The major subtypes are clear cell RCC (ccRCC) representing 65–70% of all RCC, papillary RCC (PRCC) 15–20%, and chromophobe RCC (ChRCC) 5–7%(51).

RCC is considered as an immunogenic cancer, with pathologic specimens harboring a high number of tumor-infiltrating lymphocytes (TILs) which are considered manifestations of host immune reactions against cancers(52,53).

It was suggested that approximately 30% of malignant tumor cells, including RCC among other tumors, express programmed cell death-ligand 1 (PD-L1) which closely associate with the prognosis of the patients(2,54,55). Patient prognosis depends on multiple clinicopathological factors such as TNM stage, Fuhrman nuclear grade, tumour size and other haematological indices(27). However, most of these factors correlate poorly with prognosis. Thus, there is need for new prognostication tools(56). Investigation of the role of immunological pathways in tumour progression, as well as control, has been of interest in RCC, and has led, in turn, to the development of therapeutic agents with immunological intervention points such as interferon and IL-2(57).

Nivolumab received FDA approval for the treatment of patients with advanced RCC refractory to first-line vascular endothelial growth factor (VEGF) inhibitors, demonstrating improved OS compared to everolimus (median OS 25 vs 19.6 months, HR 0.73, p = 0.002)(58). PD-L1 expression was prognostic of survival – those with higher PD-L1 expression had poorer survival than those with lower PD-L1 expression. Median OS was 21.8 months for patients with ≥1% PD-L1 expression compared with 27.4 months for patients <1% PD-L1 expression in each nivolumab-treated cohort(58). Nivolumab improved median OS in all patients compared to everolimus, regardless of PD-L1 status(59). Therefore, PD-L1

was not a reliable predictive biomarker of treatment response. An interesting observation, however, is that many poor risk and sarcomatoid tumors have high levels of PD-L1 expression in their archival tumors, and this subset of patients actually had the greatest relative benefit with nivolumab over everolimus(60,61) . These data suggest that aggressive clear cell RCC tumors upregulate PD-L1 and may be more vulnerable to checkpoint blockade.

Atezolizumab has also been investigated in mRCC. The expansion cohort of a phase Ia trial enrolled 70 patients with treatment refractory mRCC; all patients were treated with atezolizumab(62). Enrollment started with all patients regardless of PD-L1 status, but was later limited to tumors which expressed PD-L1 IC2 or IC3 (≥5% IC positive for PD-L1) by the SP142 Ventana assay. The number of patients in the trial was small but those defined as having increased PD-L1 expression had a higher ORR than those lacking PD-L1 expression (18% vs 9%).

Atezolizumab has also been investigated in the frontline setting in combination with bevacizumab, a VEGF inhibitor(63). Bevacizumab had demonstrated efficacy previously with immunotherapy, in combination with interferon alpha-2a (IFNa) among a population of untreated mRCC. The combination improved PFS in two major clinical trials, AVOREN and CALGB 90206(64,65). IMmotion 150 was a phase II trial for untreated mRCC in which patients were randomized to atezolizumab in combination with bevacizumab, atezolizumab alone, or sunitinib. Patients were allowed to crossover to the combination arm after disease progression on either atezolizumab or sunitinib. The ORR in the combination arm among PD-L1 positive patients was 46% compared to 28% in the atezolizumab arm alone, and 27% in the sunitinib arm. The hazard ratios for the combination arm compared with sunitinib were 0.64 (95%CI 0.38–1.08, p = 0.095) and 1.03 (95%CI 0.63–1.67, p = 0.917) for the atezolizumab alone vs sunitinib arm. These studies demonstrate a signal for potentially improved overall response rates for patients treated with combination therapy. Several phase III studies are currently underway investigating checkpoint inhibitors in combination with VEGF-targeted therapy for patients with mRCC(66–68).

Immunotherapy CPI combinations have proven effective in melanoma, and CheckMate-214 was the first in mRCC to use combination of CTLA-4 and PD-1 inhibitors. CHECKMATE 214 was a phase III trial which randomized 1040 patients with metastatic clear cell RCC to treatment with either combination nivolumab- ipilimumab or sunitinib. Co-primary endpoints

included ORR, progression free survival (PFS), and OS, specific- ally in the IMDC intermediate or high risk population. Secondary endpoints included PFS and OS for the intention to treat population (including favorable risk). Nivolumab-ipilimumab improved both median OS (not reached (NR) vs 26.0 months, HR 0.63, $p < 0.0001$) and ORR (42% vs. 27%, $p<0.0001$) in patients with intermediate-high risk disease [25]. In the IMDC intermediate or high risk patients, ORR was 37% in PD-L1 negative patients 58% and PD-L1 positive patients(69). In the PD-L1 negative patients with IMDC intermediate or high risk, PFS did not differ between those treated with nivolumab-ipilimumab versus sunitinib (HR 1.00, $p= 0.98$), whereas in PD-L1 positive population, there was a large difference in PFS between these two groups (HR 0.48, $p = 0.0003$)(69). However, both PD-L1 positive and PD-L1 negative patients benefited with improved overall survival. Therefore, PFS was not a good surrogate endpoint for survival benefit in the PD-L1 negative cohort. Given the small difference in response rates in PD-L1 positive versus PD-L1 negative patients, as well as the improvement in mOS for these patients, the role for PD-L1 testing remains unclear – negative PD-L1 status would not necessarily select patients who would not benefit from nivolumab-ipilimumab. Indeed, in the IMDC favorable risk group, ORR favored sunitinib over nivolumab-ipilimumab (52% vs. 29%, $p = 0.0002$)(69). Further data needs to be presented regarding PD-L1 status in patients with favorable risk disease, and their survival analyses. For now, however, PD-L1 status is not clinically useful in informing treatment decisions in mRCC.

Further correlative work has also emphasized the ineffectiveness of PD-L1 as a predictive biomarker in mRCC. Primary tumor and metastatic tumors have discordant PD-L1 expression – in one pathology-based study, discordant PD-L1 expression was detected in 21% (11/53) of cases, suggesting that analysis of metastatic biopsies may be necessary to form an accurate assess- ment of PD-L1 expression(70). Moreover, PD-L1 expression is dynamic and can arise after treatment as a form of treatment resistance(71). This further emphasizes the inadequacy of archival tissue to assess such a dynamic biomarker.

Journey of Immune checkpoint inhibitors in urothelial carcinoma

Currently, multiple immune checkpoint inhibitors including anti-PD-1 and anti-PD-L1 have been approved in metastatic and advanced urothelial carcinoma expanding the scope of treatment of urothelial carcinoma. Identifying patients who may or may not respond to PD1/PDL1 inhibitors is important as the majority of patients in different clinical trials did not

have an overall response(72). Although five immune checkpoint inhibitors have been approved in urothelial carcinoma, only two companion diagnostic immunohistochemistry assay has been approved by the US FDA(73).

Urothelial carcinoma and PD-L1 testing

The phase I study of atezolizumab in mUC was initially designed to include PD-L1-positive enriched cohorts, with a dose expansion cohort for all mUC patients regardless of PD-L1 status(74). Forty-three percent (13/30) of patients with a positive PD-L1 tumor had an objective response to atezolizumab compared with only 11% (4/35) of patients with negative PD-L1 status, suggesting that PD-L1 IHC status might predict treatment response. Following these results, several studies were conducted to confirm the anti-tumor activity of PD-L1 and PD-1 inhibitors in two distinct populations: patients with mUC who had progressed after platinum-based therapies and patients with mUC who were not candidates for first-line platinum-based therapies.

Post-platinum mUC population

IMvigor 210 (Cohort 2) and KEYNOTE-045 explored the use of atezolizumab and pembrolizumab, respectively, in the post-platinum mUC population. IMvigor 210 enrolled patients with locally advanced or mUC refractory to cisplatin-based chemotherapy. While the objective response rate (ORR) of the entire cohort was 15%, the ORR was 26% (26/100) in PD-L1 positive patients, compared with only 9% (19/210) in PD-L1 negative patients. These results seemed to confirm earlier studies showing the potential for PD-L1 as a predictive marker in mUC. Based on these results the Phase III IMvigor 211 trial randomized patients to atezolizumab or chemotherapy (paclitaxel, docetaxel or vinflunine)(75) with a primary endpoint of overall survival (OS) in PD-L1 positive subjects. The secondary endpoint of OS in the intention-to-treat (ITT) population was analyzed after the initial subset of PD-L1 positive cohort. While the ORR for the PD-L1 enriched cohort was 23% compared with 13% in the ITT cohort and confirmed prior findings, somewhat surprisingly, for the high PD-L1 cohort there was no statistical difference in mOS when comparing atezolizumab to single agent chemotherapy (HR: 0.87; OS: 11.1 vs 10.6 months; $p = 0.41$)(75). Interestingly, a significant difference in OS was observed in the ITT analysis for all patients treated with atezolizumab vs chemotherapy (HR: 0.85; OS: 8.6 vs 8.0 m; $p = 0.038$). Given these somewhat contradictory results, the accelerated FDA-approval for atezolizumab in mUC did

not change after the results of IMvigor 2011, and further investigation of atezolizumab is underway in a Phase III study of platinum-naïve mUC patients. Based on these findings, it may be premature to select patients for therapy in clinical trials of CPIs based on the Ventana SP142 assay.

PD-L1 status did not predict for response in KEYNOTE- 045(76), a phase III trial which randomized 542 patients with mUC to treatment with either pembrolizumab or standard of care chemotherapy. KEYNOTE-045 utilized the 22C3 mouse antibody IHC assay (Dako/Agilent, CA, USA) using a combined positive score (CPS) to define PD-L1 positivity. The CPS was calculated as the percentage of PD- L1 expressing tumor and infiltrating immune cells relative to the total number of tumor cells; CPS ≥10% was considered PD-L1 positive. The ORR for all patients was 22%, and there was no difference between patients with CPS ≥ 10% compared with patients with a CPS < 10%. Thus, CPS was not predictive of response to treatment with pembrolizumab in this patient population. This result was in direct contrast to the data in non-small cell lung cancer (NSCLC), where CPS ≥ 50% does correlate with response to pembroli- zumab(77). More importantly, PD-L1 appeared to be a marker of poor prognosis, with patients who were PD-L1+ having poorer outcomes compared to patients who were PD-L1 negative by the CPS score, regardless of whether they received chemotherapy or pembrolizumab.

Three additional CPIs have now been studied in and received accelerated FDA approval for the platinum- refractory population of mUC – nivolumab, durvalumab, and avelumab(78–80). In the CheckMate 275 phase II trial of nivolumab, PD-L1 status was determined using the 28–8 rabbit antibody (Dako/Agilent, CA, USA). PD- L1 positive patients had a ORR of 28% (23/81) compared with an ORR of 16% (29/184) for PD-L1 negative patients(80). For durvalumab and avelumab, the ability to predict the responders was somewhat greater, but in smaller, earlier phase studies. For durvalumab, a positive PD-L1 status (using the SP263 rabbit antibody, Ventana, AZ, USA) was defined as ≥25% of tumor cells or tumor infiltrating immune cells expressing PD-L1. Patients who met this definition had an ORR of 18% (27/98), compared with 5% (4/79) of patients who had a negative PD-L1(81). For avelumab, a positive PD-L1 status was defined as >5% PD-L1 expression by 73–10 rabbit antibody IHC (Dako/Agilent, CA, USA) on tumor cells(79). The ORR was 54% (7/13) in the PD-L1 positive group, compared with 4% (1/24) in the PD-L1 negative group. While the use of PD-L1 as a predictive biomarker looks promising in these two studies, these were small studies,

using different PD-L1 assays with different thresholds defining PD-L1 positivity. The difference in the prevalence of PD-L1 positivity across the trials suggests that different populations of "PD-L1 positive" patients are being captured by the different assays, which is further complicated by the different thresholds for positivity.

For example, in the durvalumab trial, 51% of patients were defined as PD-L1 positive, compared with only 16% of patients in the avelumab trial(79,81). This underscores the complexity of attempting cross-trial comparisons. In addition to different PD-L1 criteria, this may also be due to different inclusion criteria – the avelumab trial required at least one previous line of treatment but the durvalumab trial did not. As clinical development moves forward for each of these agents, further assay standardization, test characteristics, definitions of the "biomarker-positive" population all need to be addressed.

Platinum ineligible mUC

Due to a variety of factors, including renal or hearing impairment, poor performance status, and neuropathies, 30% to 50% of patients with chemotherapy-naïve advanced UC are not candidates for platinum-based chemotherapy(82). Cohort 1 of IMvigor 210 and KEYNOTE-052 explored the use of atezolizumab and pembrolizumab, respectively, in platinum-ineligible patients with mUC. Using the SP142 Ventana assay, PD-L1 positive patients in IMvigor 210 had an ORR of 28% (9/32) compared to 20% (18/87) in those who were PD-L1 negative(83). Thus, the difference in ORR between PD-L1 positive and PD-L1 negative patients was minimal. In KEYNOTE-052, PD-L1 (with the 22C3 assay) appeared to be associated with higher response rates: 51% (41/80) of patients with a CPS $\geq$ 10% had an objective response compared with 23% (42/185) of patients with a CPS < 10%(84). It should be emphasized that these studies in chemotherapy-naïve, cisplatin ineligible patients were both single-arm phase II studies, and assessment of the predictive capacity of PD-L1 would be better explored in appropriately powered Phase III studies. The disparate results again suggest that a single PD-L1 score is not sufficient to predict the population of patients who will respond to immune CPIs. Multiple Phase III studies are underway (JAVELIN bladder 100 with avelumab [NCT02603432], IMvigor 130 with atezolizumab [NCT02807636], and DAN- UBE with durvalumab and tremelimumab [NCT02516241]) and will further explore the predictive and prognostic capacity of PD-L1.

Based on the data presented above, in which patients with mUC may respond to PD-1/PD-L1 blockade even if their archival tumor lacks PD-L1 expression, we do not recommend PD-L1 clinical testing in UC patients. While in some studies PD-L1 positivity may identify patients more likely to have an objective response, and combined tumor/microenvironment testing may further enrich for responders, in others studies this biomarker has no discriminatory power, and given the conflicting results treatment with a CPI should not be withheld based on PD-L1 status in mUC. Prospective studies of PD-L1 as a predictive biomarker are needed, with consideration for contemporary/recent biopsies, tumor heterogeneity assessments, and expression in tumor vs normal immune cells.

AIM & OBJECTIVES

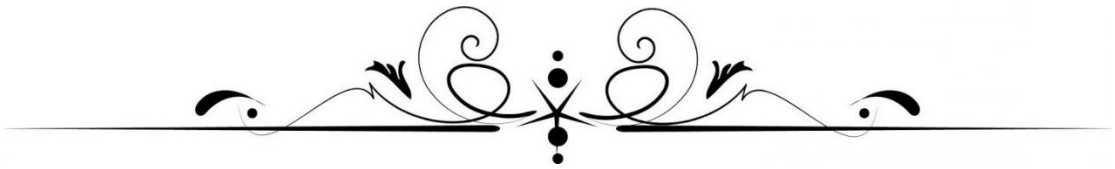
AIM AND OBJECTIVES

Primary objectives

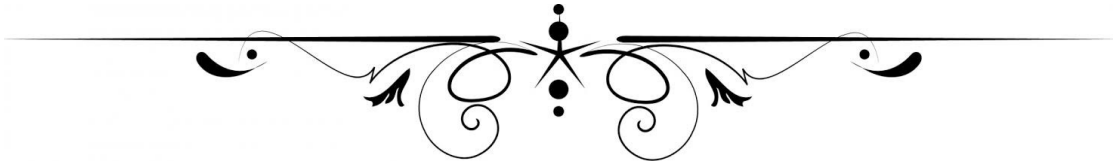
1. To find out the association of PD-L1 expression in patients with genitourinary malignancies and clinical parameters of the patients
2. To find out the association of PD-L1 expression in patients with genitourinary malignancies and radiological and pathological (where available) stage of tumor

Secondary objectives

1. To find out the association of PD-L1 expression in patients with genitourinary malignancies and pathological grade of tumor



MATERIALS & METHODS



MATERIALS AND METHODS

PLACE OF STUDY: This study was conducted from December 2020 to November 2022 at the Department of Urology and Department of Pathology, All India Institute Of Medical Sciences, Jodhpur, Rajasthan, India.

STUDY DESIGN: Prospective Observational Study

PATIENT SELECTION:

INCLUSION CRITERIA: All patients diagnosed with genitourinary mass and undergoing surgery or biopsy for the same and diagnosed with malignancy on histopathological examination (HPE).

EXCLUSION CRITERIA:

1) Benign pathology on HPE
2) Patients not willing to participate in the study
3) Penile malignancy

METHODOLOGY

Following parameters were assessed

1) Clinical parameters: age, gender, Body Mass Index (BMI), smoking or other addiction, comorbidities and other demographic details
2) Laboratory parameters: complete blood count (CBC), kidney function test (KFT), serum electrolytes (SE), liver function test (LFT)
3) Radiological findings: ultrasound abdomen and pelvis, computed tomography (CT scan), magnetic resonance imaging (MRI) or positron emission tomography (PET)
4) Histopathologic assessment: formalin fixed paraffin embedded tissue of genitourinary mass will be assessed for:
    a) Tumor type
    b) Tumor staging
    c) Tumor grade
    d) Immunohistochemical assay for PD-L1

Tissue processing and staining

All the biopsies were fixed in 10% neutral buffered formalin. After sectioning, tissue was processed as follows:

1. Dehydration was carried out by passing the sections through a series of ascending grades ofethyl alcohol, from 50%, 70%, 95% to absolute alcohol.
2. The clearing was done by passing the tissue through two changes of xylene.
3. Impregnation was done in molten paraffin wax which had a melting point of 54 – 62˚C.
4. Embedding: Embedding station (Leica EG 1150 H) was used through which a small amount of liquid paraffin was layered into aluminum molds. Properly oriented tissues were placed inside the molds, which were then filled with liquid paraffin 60 – 62˚C and allowed to cool and harden. The lower portion of the cassette with an identification number was used as the final block.
5. Microtomy: Microtome (Leica-RM2255) was used and thin ribbons (4-5 μm) were cut and floated in warm water (~56˚C) for expansion of the curled sections. These sections were then collected on frosted glass slides and kept for drying.

1) Staining of sections: (for H and E stain)

1. Deparaffinization – The glass slides containing the tissue sections were kept over the hot plate at 60 ˚C for 10 minutes, followed by two changes in xylene (Xylene I & Xylene II), 10 minutes each.
2. Hydration – Through graded alcohol (100%, 95%, 70%, 50%) to water, 10 minutes respectively.
3. Hematoxylin – The sections were kept in Harris's Hematoxylin for 5 minutes.
4. Washing – The sections were washed well in water for 2 minutes.
5. Differentiation – Done in 1% acid alcohol (1% HCl in 70% alcohol) for 10 seconds.
6. Washing – Done under running tap water (usually for 15 – 20 minutes) until the sections 'blue'.
7. Eosin – Stained in 1% Eosin Y for 10 seconds.
8. Washing – Done in running tap water for 2 minutes.
9. Dehydration – Through graded alcohol (50%, 70%, 95%, 100%), 10 minutes each.
10. Clearing –Through xylene (Xylene II & Xylene I), 2 minutes each.
11. Mounting – The sections were mounted in DPX with a coverslip.

2) Immunohistochemistry antibodies used :

Primary antibody:
Ready to use.

For identifying programmed death-ligand status PD-L1 antibody was used.

- PD-L1 (Programmed death-ligand 1: Prediluted, Clone: CAL10, Company: Biocare Medical)

Secondary Antibody: Bond Polymer Refine Detection, Leica

- Peroxide block, 3-4%(v/v)
- Post Primary, Rabbit anti-mouse IgG in 10% (v/v) animal serum in tris-buffered saline
- Polymer, Anti-rabbit Poly-HRP-IgG containing 10% (v/v) animal serum in tris-bufferedsaline
- DAB Part 1, in stabilizer solution
- DAB Part B ≤0.1% (V/V) Hydrogen peroxide in stabilizer solution
- DAB Part B ≤0.1% (V/V) Hydrogen peroxide in stabilizer solution
- Hematoxylin, 0.1%

Steps of IHC staining:

A. Preparation of Buffer–Two types of buffers were used.

1. Wash Buffer
2. Antigen Retrieval Buffer (ARB)

Wash buffer preparation: 6 gm powdered TRIS buffer salt was dissolved into 1 liter of distilled water and pH was set at 7.4.

ARB preparation: 6.05 gm TRIS salt and 0.744 gm EDTA salt were dissolved in 1 liter ofdistilled water, pH was set at 9.0.

Note:

- To increase the pH, NaOH solution was added drop by drop and pH was titrated.
- To decrease the pH, HCl was added drop by drop and pH was titrated.

B. Preparation of Poly-L-Lysine Solution (PLL Solution):

1 ml of PLL was diluted with 9 ml of distilled water (1 in 10 dilutions).

C. Slide Coating Procedure:

Step 1: Diluted PLL solution was taken in a clean container/Coplin jar Step 2: Both sides of the glass slides were cleaned with tissue paper Step 3: The clean slides were immersed in a PLL solution for 5 minutes

Step 4: After 5 minutes, the coated slides were removed and kept overnight for air drying. Thecoated slides were kept at room temperature. Tissue sections of 4 µ thickness were obtainedon the PLL coated slides.

Baking: The slides were kept at 60˚C for 1 hour and then cooled to room temperature.

IHC staining procedure

Step 1: Deparaffinization – The slides were kept in Xylene I (10 minutes), followed byXylene II (10 minutes).

Step 2: Rehydration – The slides were kept in 100%, 70%and 50% alcohol for 5 minutes eachfollowed by running tap water for 5 minutes.

Step 3: Antigen retrieval – by pressure cooker method [38]. 200 ml of clean tap water wastaken in the empty pressure cooker and heated up to the steam formation. The slides were placed in a rack. 300 ml of ARB was put in the container and the rack with slides was placed inside the container. Then the container containing the rack with slides, was placed inside the pressure cooker and the lid was closed. After two whistles the pressure was released by liftingthe air vent and allowed to cool till it reached room temperature.

Step 4: Wash – Slides were washed in Wash Buffer (pH7.4) thrice at a 1-minute interval.

Step 5: Peroxide blocking – Blocking reagent was added to the sections and incubated for 10 minutes in the Humidity chamber at room temperature. This step prevents unwanted, non-specific background staining.

Step 6: The peroxide was decanted and not washed with buffer.

Step 7: Primary antibody – PD-L1, was added to thesections and incubated in the Humidity

chamber for one hour.

Step 8: Wash – After that slides were washed in Wash Buffer (pH 7.4) thrice at a 1-minuteinterval.

Step 9: Amplifier – Amplifier was added over the sections and incubated for 30 minutes in theHumidity chamber at room temperature.

Step 10: Wash – The slides were washed in Wash Buffer (pH 7.4) thrice at a 1-minute interval.

Step 11: HRP label – The HRP was added and incubated for 30 minutes in the Humiditychamber at room temperature.

Step 12: Wash – The slides were washed in Wash Buffer (pH 7.4) thrice at a 1-minute interval.

Step 13: DAB – The DAB chromogen was applied to the sections and incubated in theHumidity chamber for 10 minutes, avoiding light exposure as much as possible.

Step 14: Wash – The sections were washed in distilled water twice at a 1-minute interval.

Step 15: Counterstain – Slides were counterstained using Harris Haematoxylin for 2-3 minutes.Step 16: Wash – The slides were washed in running tap water for 5 minutes.

Step 17: Dehydration – was done in graded alcohol (50%, 70%, 95%, 100%), 1 minute each.

Step 18: Mounting – Slides are air-dried, mounted with DPX and examined under the microscope.

Interpretation of immunohistochemical stain for Programmed Death- Ligand 1 (PD-L1)

The H&E stained slide and the PD-L1 slides were examined on microscope (Nikon, model Eclipe Ci-L). The percentage of tumour infiltrating lymphocytes (TILs) were assessed on H&E stained sections and the Combined Positive Score (CPS) and Tumour Proportion Score (TPS) were calculated on the PD-L1 immunohistochemistry slides. Expression of PD-L1 in the tumour was quantified manually and classified as positive when staining (PD-L1: membranous) was present in ≥1% of tumour cells, and specifically when the CPS was >1.

Combined positive score (CPS)

The combined positive score was determined manually and was based on the equation described previously for gastric and gastroesophageal junction cancers.

CPS = [(number of PD-L1-positive tumour cells and mononuclear inflammatory cells)/(total

number of tumour cells)].

In the CPS system, immune cell scoring is based on PD-L1-positive lymphocytes and macrophages (‘mononuclear inflammatory cells’) identified in association with a tumoural immune response.

This includes both intratumoural immune cells and peritumoural immune stromal cells, but not the immune cells in stroma distant from the tumour.

Tumour Proportion Score (TPS): Percentage of tumour cells expressing PD-L1.

STATISTICAL ANALYSIS

Categorical variables were presented in number and percentage (%) and continuous variables will be presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test.

1) Quantitative variables was compared using t-test (parametric)/ Mann Whitney test (non parametric) across follow ups
2) Qualitative variables was compared using Chi-Square test/ Fisher's exact test.

A p value of <0.05 was considered significant.

The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package For Social Sciences (SPSS) version 21.0

Type of study: Prospective Observational Study.

SAMPLE SIZE: Since the study was time bound, we included all cases fulfilling the inclusion criteria between December 2020 to November 2022.

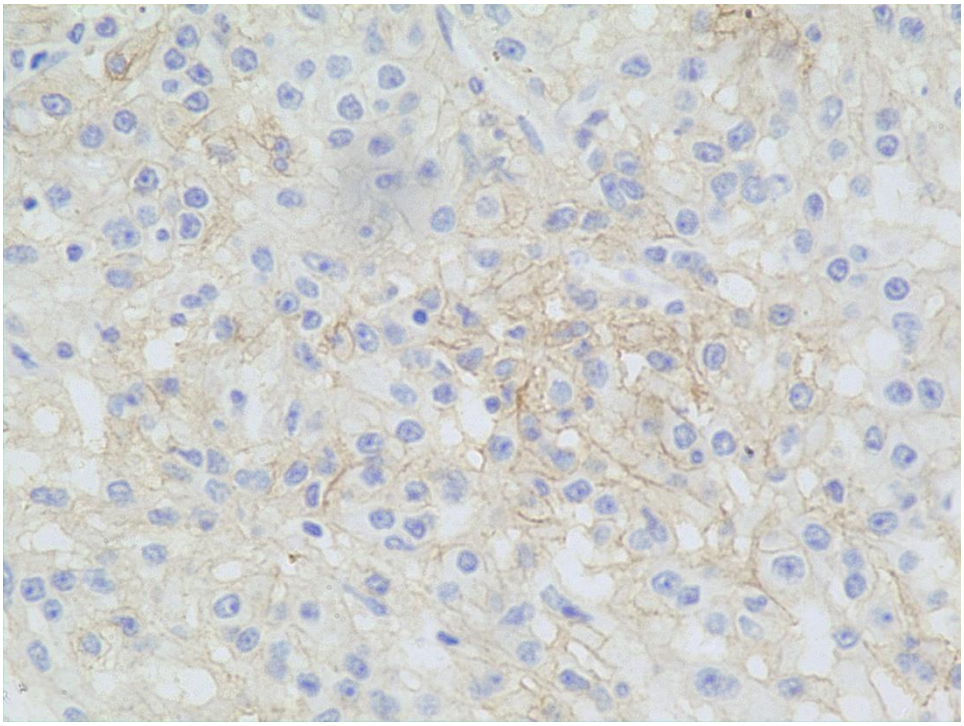


Figure 3: A case of clear cell renal cell carcinoma (RCC) showing 80% of the tumour cells expressing PD-L1

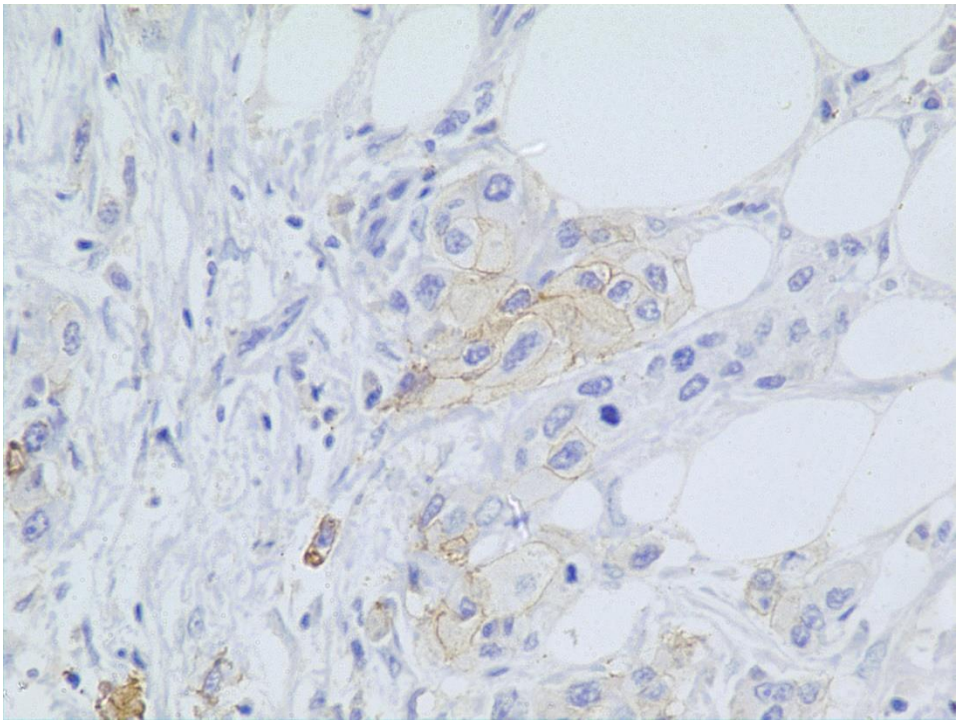


Figure 4: A case of invasive high grade urothelial carcinoma involving the perivesical soft tissue and expressing PD-L1. Adjacent adipose tissue is also seen in this image.

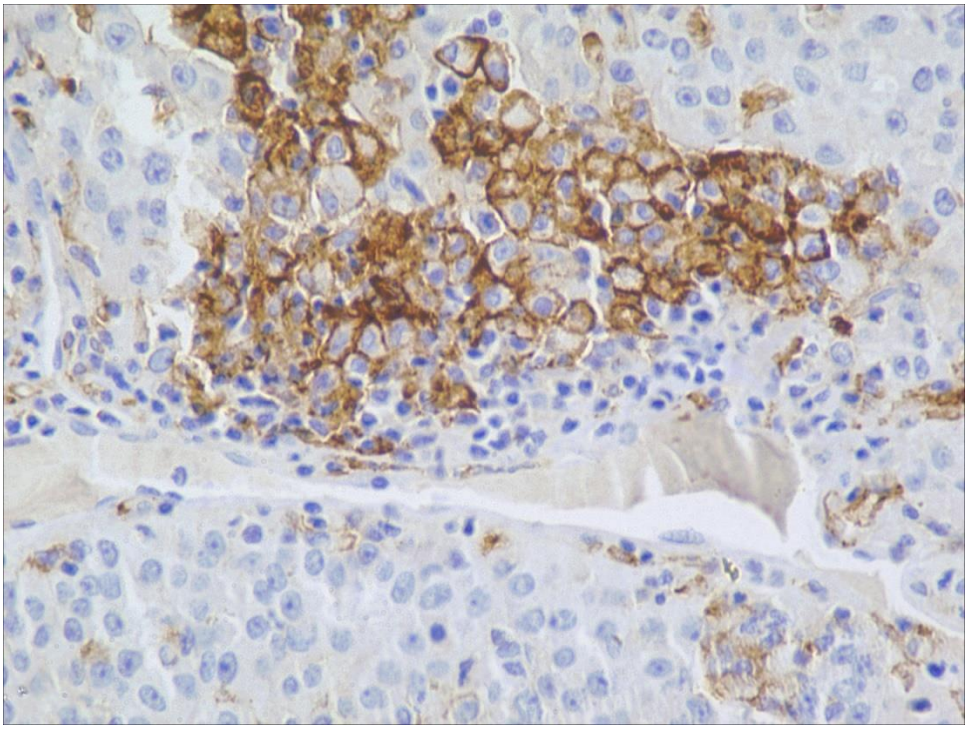


Figure 5: A case of clear cell renal cell carcinoma (RCC) expressing PD-L1 in the tumour cells as well as in the TILs.

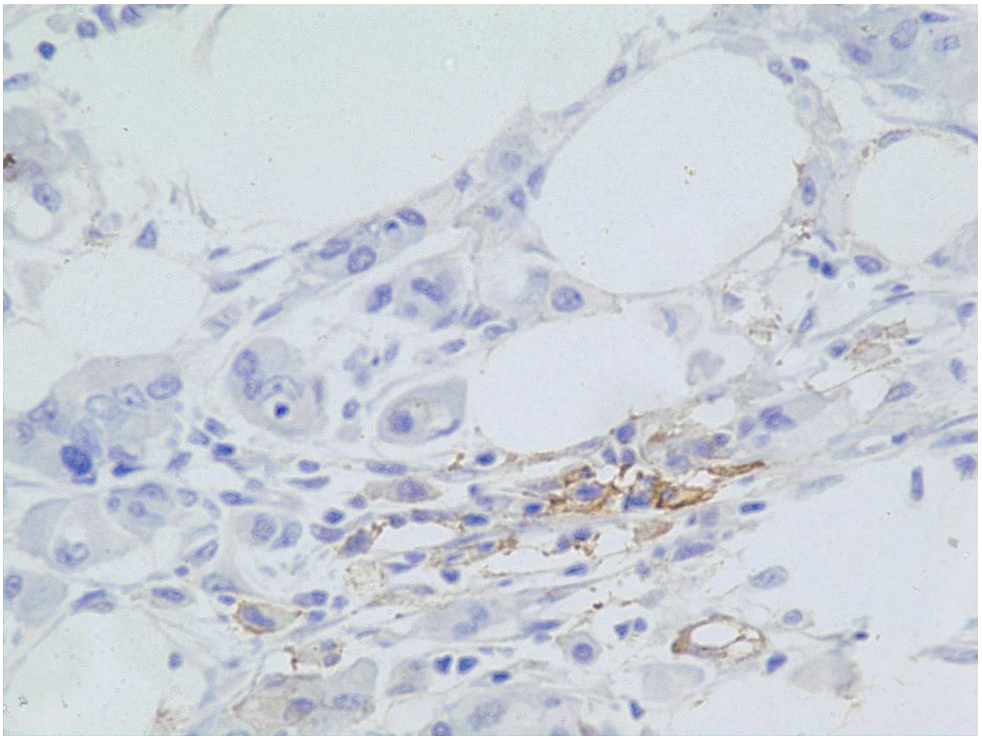


FIG 6: A case of invasive high grade urothelial carcinoma involving the perivesical soft tissue, with expression of PD-L1 in the tumour infiltrating lymphocytes (TILs). Adjacent adipose tissue is also seen in this image.

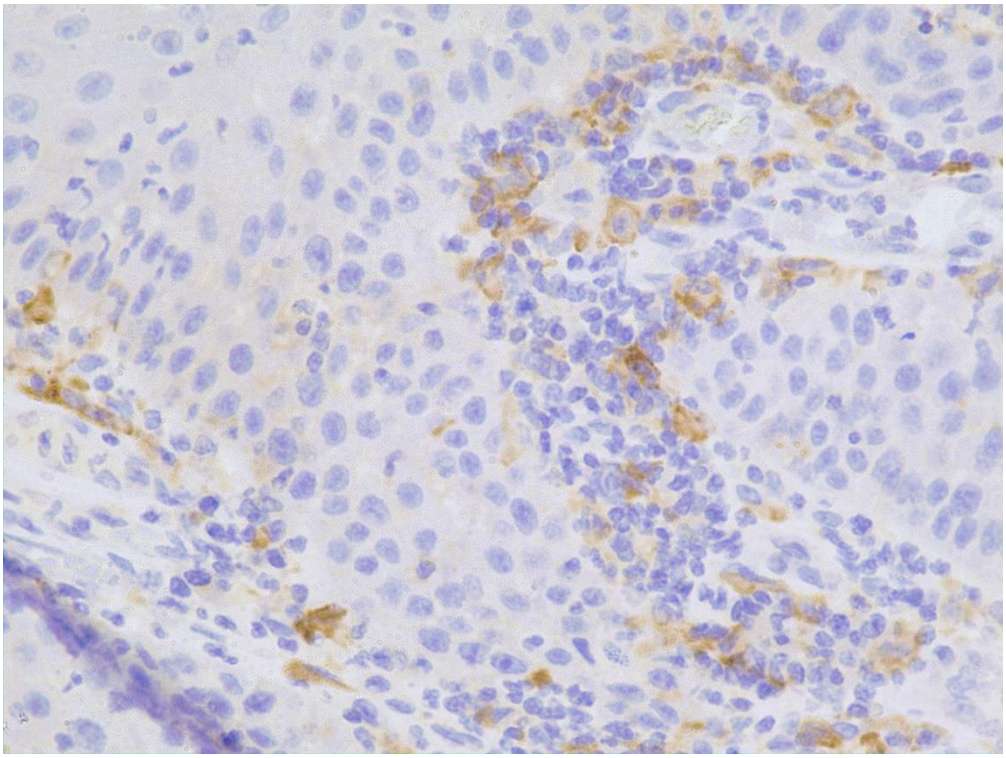


Figure 7: A case of invasive urothelial carcinoma expressing PD-L1 predominantly in the TILs, with focal PD-L1 positivity in the tumour cells.

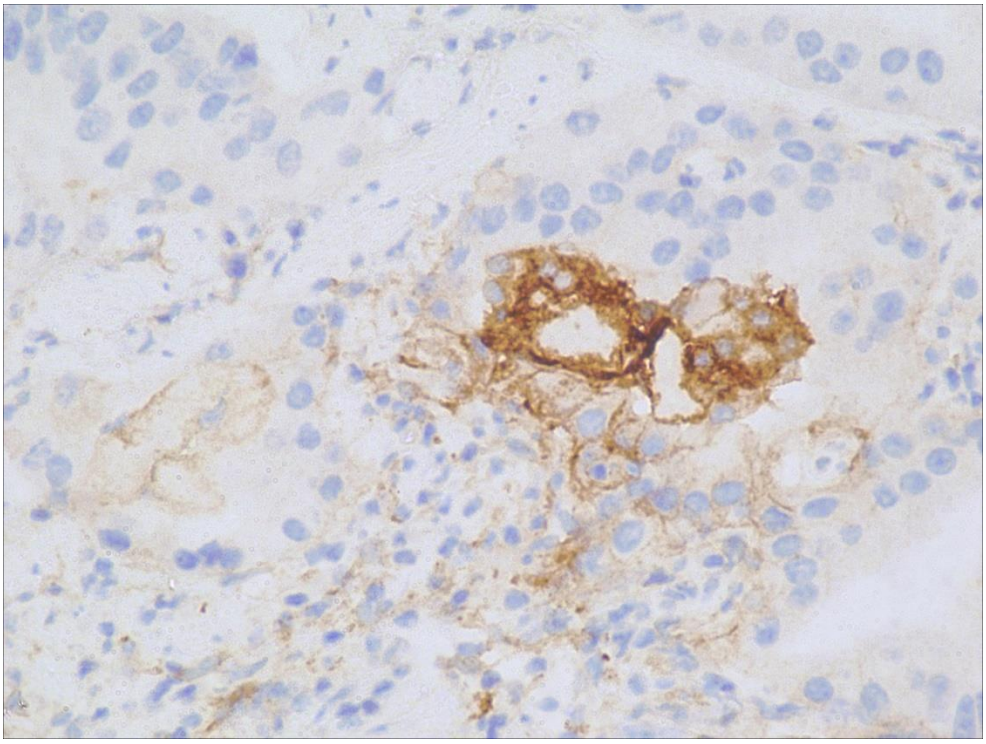


Figure 8: A case of acinar adenocarcinoma of prostate expressing PD-L1 in the tumour cells as well as in the TILs.

RESULTS

RESULTS

Table 1: Total number of various malignancies in study population

	Frequency	Percent
BLADDER	38	39.2
KIDNEY	30	30.9
PROSTATE	29	29.9
Total	97	100.0

Our study included 97 patients- 38 of Bladder carcinoma, 30 of Renal cell carcinoma and 29 patients of Prostate carcinoma accounting for 39.2%, 30.9%, and 29.9% of total cases respectively.

Table 2: Mean, median age of study population

ORGAN	N	Minimum	Maximum	Mean	Std. Deviation	Median
BLADDER	38	28	82	55.16	14.494	54.50
KIDNEY	30	21	73	49.17	13.722	51.00
PROSTATE	29	50	86	66.28	9.399	66.00
Total	97	21	86	56.63	14.491	58.00

As shown in the table above, total number of cases were 97 with median age of 54.50 years, 51 years, 66 years in Bladder, Renal, Prostate cancer respectively.

Table 3: PD-L1 status in study population

	PD-L1 positive	PD-L1 negative	Total
Prostate cancer	12(41.4%)	17(58.6%)	29(100%)
Renal cancer	18(60%)	12(40%)	30(100%)
Urothelial cancer	27(71.1%)	11(28.9%)	38(100%)

Out of 29 patients of prostate cancer, PD-L1 expression was seen in 41.4% patients. In Renal and Urothelial cancer, the PD-L1 positivity rate was 60%, 71.1% respectively. PD-L1 expression is comparatively higher in urothelial cancer as shown in table 3.

Figure 9: PD-L1 status in study population

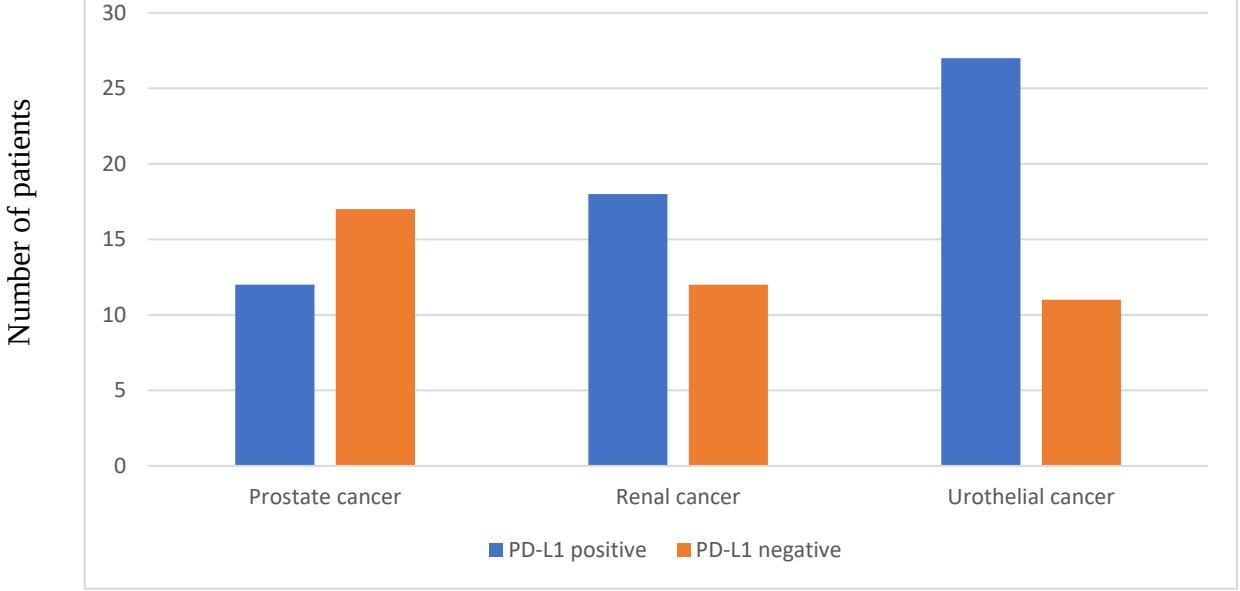


Table 4.1: Prostate carcinoma : Patients' characteristics

Characteristics	
Age (years)	N=29
≤70	18(62.1%)
>70	11(37.9%)
ECOG performance status	
0	10(34.5%)
1	10(34.5%)
2	4(13.8%)
3	3(10.3%)
4	2(6.9%)
Gleason score	
<7	5(17.2%)
7	10(34.5%)
≥8	14(48.3%)
PSA(ng/ml)	
<4	2(6.9%)
4-10	6(20.7%)
10-20	7(24.1%)
>20	14(48.3%)
Perineural invasion	
Present	17(58.6%)
Absent	12(41.4%)
Lymphovascular invasion	
Present	4(13.8%)
Absent	25(86.2%)

Our study included 29 patients of Prostate cancer with median age of 66 . Most of the patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0,1 at presentation. Most patients had PSA>20 (48.3%) and perineural invasion was seen in 58.6% (17) of patients as shown in table 4.1.

Table 4.2: Prostate carcinoma : Relationship between PD-L1 expression and clinicopathological factors

Characteristics	**PD-L1 Expression positive**	**PD-L1 Expression negative**	**P-value**
Age (years) N=29			
≤70	5(41.7%)	13(76.5%)	0.119[$]
>70	7(58.3%)	4(23.5%)	
ECOG performance status			
0	4(33.3%)	6(35.3%)	0.991[#]
1	4(33.3%)	6(35.3%)	
2	2(16.7%)	2(11.8%)	
3	1(8.3%)	2(11.8%)	
4	1(8.3%)	1(5.9%)	
Gleason score			
<7	3(25.0%)	2(11.8%)	0.635[#]
7	4(33.3%)	6(35.3%)	
>7	5(41.7%)	9(52.9%)	
PSA(ng/ml)			
<4	1(8.3%)	1(5.9%)	0.771[#]
4-10	2(16.7%)	4(23.5%)	
10-20	4(33.3%)	3(17.6%)	
>20	5(41.7%)	9(52.9%)	
Perineural invasion			
Present	8(66.7%)	9(52.9%)	0.703[$]
Absent	4(33.3%)	8(47.1%)	
Lymphovascular invasion			
Present	3(25.0%)	1(5.9%)	0.279[$]
Absent	9(75.0%)	16(94.1%)	

$-Fisher's Exact Test

#-Chi square Test

The PD-L1 expression and in tumor cells and their correlation with clinicopathological factors is summarised in table 4.2. The results showed that tumor PD-L1 expression was not related to age, ECOG status, Gleason score, PSA, perineural invasion. Lymphovascular invasion was seen in 25% (3 out of 12) in PD-L1 expressing tumor whereas it was seen in 5.9% (1 out of 17) of PD-L1 negative tumors. However, it was not found to be statistically significant.

Figure 10: Prostate carcinoma : Relationship between PD-L1 expression and clinicopathological factors

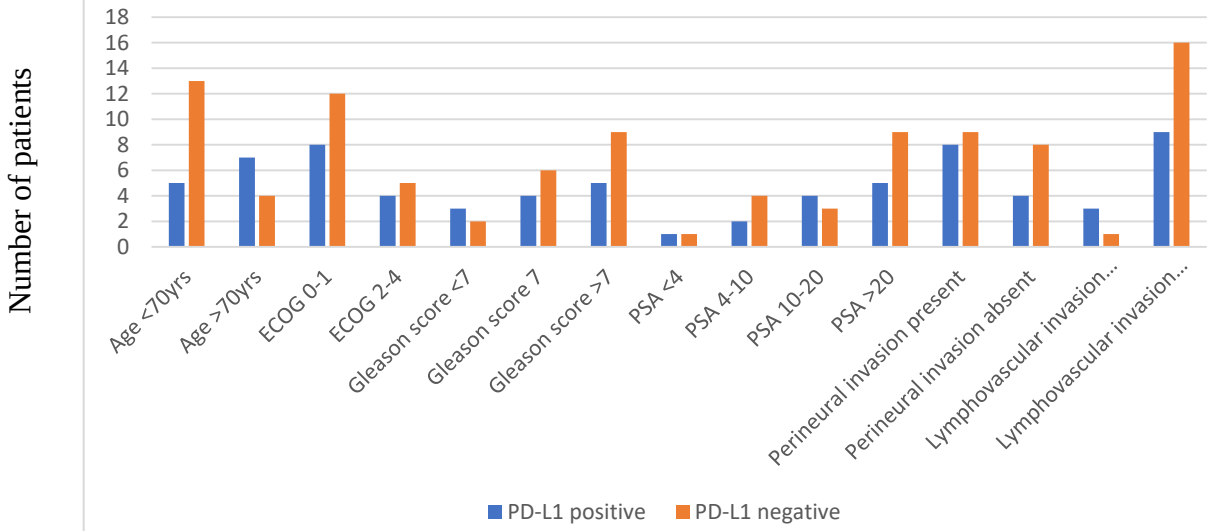


Table 5.1: Renal Cell Carcinoma : Patients' characteristics

Characteristics	
Age (years)	N=30
≤50 >50	15(50%) 15(50%)
Gender	
Male Female	8(26.7%) 22(73.3%)
ECOG performance status	
0 1 2 3 4	16(53.3%) 4(13.3%) 6(20.0%) 3(10.0%) 1(3.3%)
Histological subtype	
Clear cell Papillary Chromophobe Oncocytic	19(63.3%) 7(23.3%) 2(6.7%) 2(6.7%)
Nuclear grade	
Grade 1 Grade 2 Grade 3 Grade 4	8(26.7%) 13(43.3%) 7(23.3%) 2(6.7%)
Pathological stage pT stage	
T1 T2 T3 T4	8(26.7%) 10(33.3%) 10(33.3%) 2(6.7%)
pN stage	
N0 N1 Nx	6(20%) 4(13.3%) 20(66.7%)

This study included 30 patients with tissue diagnosis of RCC.

Patients had a median age of 54.5 years with a female predominance (73.3%). Most of the patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 at presentation. Most common histological subtype was Clear cell RCC (63.3%). Patients' characteristics are shown in table 5.1

Table 5.2: Renal cell carcinoma: Relationship between PD-L1 expression and clinicopathological factors

Category	PD-L1 Expression positive	PD-L1 Expression negative	P-value
Age (years) N=30			
≤50	10(55.6%)	5(41.7%)	0.710[$]
>50	8(44.4%)	7(58.3%)	
Gender			
Male	4(22.2%)	4(33.3%)	0.678[$]
Female	14(77.8%)	8(66.7%)	
ECOG performance status			
0	8(44.4%)	8(66.7%)	0.572[#]
1	2(11.1%)	2(16.7%)	
2	5(27.8%)	1(8.3%)	
3	2(11.1%)	1(8.3%)	
4	1(5.6%)	0(0%)	
Histological subtype			
Clear cell	10(55.6%)	9(75.0%)	0.471[#]
Papillary	6(33.3%)	1(8.3%)	
Chromophobe	1(5.6%)	1(8.3%)	
Oncocytic	1(5.6%)	1(8.3%)	
Nuclear grade			
Grade 1	5(27.8%)	3(25.0%)	0.987[#]
Grade 2	8(44.4%)	5(41.7%)	
Grade 3	4(22.2%)	3(25.0%)	
Grade 4	1(5.6%)	1(8.3%)	
Pathological stage pT stage			
T1	5(27.8%)	3(25.0%)	0.816[#]
T2	7(38.9%)	3(25.0%)	
T3	5(27.8%)	5(41.7%)	
T4	1(5.6%)	1(8.3%)	

pN stage N=10			
N0 N1	2(66.7%) 1(33.3%)	4(57.1%) 3(42.9%)	>0.99[$]

$-Fisher's Exact Test

#-Chi square Test

In correlation with clinical and pathological factors, patients with PDL1 positive tumor cells had comparable results with PD-L1 negative tumors, as presented in table 5.2 . It showed that PD-L1 expression was not related to age, gender, ECOG status, nuclear grade, T stage, Lymph node metastasis, histological subtype.

Figure 11: Renal cell carcinoma: Relationship between PD-L1 expression and clinicopathological factors

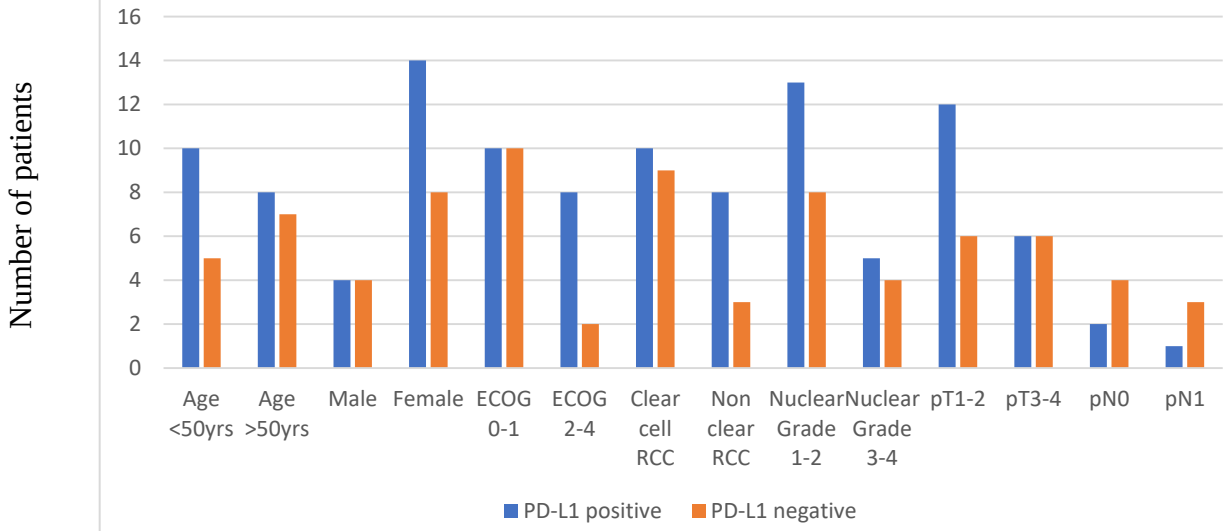


Table 6.1: Urothelial carcinoma : Patients' characteristics

Characteristics	
Age (years)	N=38
≤50	15(39.5%)
>50	23(60.5%)
Gender	
Male	32(84.2%)
Female	6(15.8%)
ECOG performance status	
0	15(39.5%)
1	9(23.7%)
2	7(18.4%)
3	5(13.2%)
4	2(5.3%)
Histological grade	
High	21(55.3%)
Low	17(44.7%)
Muscle Invasion	
Present	15(39.5%)
Absent	23(60.5%)

Our study consisted of 38 patients. The clinicopathological characteristics are shown in table 6.1. 60.5% (23) of cases were aged >50 years with male predominance (84.2%).

Table 6.2: Urothelial carcinoma : Relationship between PD-L1 expression and clinicopathological factors

Characteristics	PD-L1 Expression positive	PD-L1 Expression negative	P-value
Age (years) N=38			
50	9(33.3%)	6(54.5%)	0.285$
>50	18(66.7%)	5(45.5%)	
Gender			
Male	23(85.2%)	9(81.8%)	>0.99$
Female	4(14.8%)	2(18.2%)	
ECOG performance status			
0	12(44.4%)	3(27.3%)	0.845#
1	6(22.2%)	3(27.3%)	
2	5(18.5%)	2(18.2%)	
3	3(11.1%)	2(18.2%)	
4	1(3.7%)	1(9.1%)	
Histological grade			
High	18(66.7%)	3(27.3%)	0.037$
Low	9(33.3%)	8(72.7%)	
Muscle Invasion			
Present	13(48.1%)	2(18.2%)	0.145$
Absent	14(51.9%)	9(81.8%)	

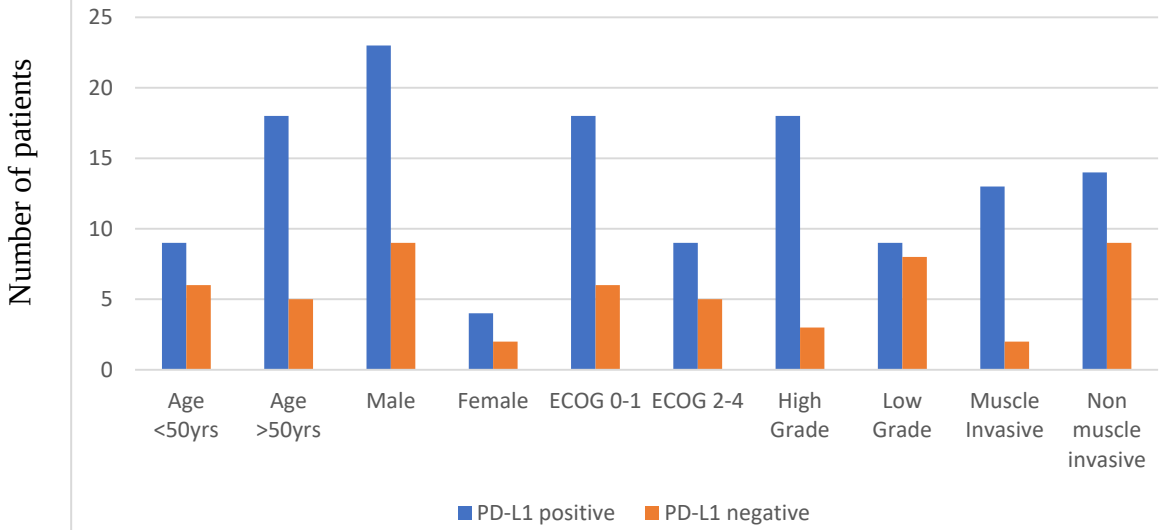
$-Fisher's Exact Test

#-Chi square Test

PD-L1 expression in tumor cells was noted in 71.1% (27 out of 38) of cases.

Immunoexpression of PD-L1 showed no statistical difference in age, gender, ECOG performance status of the patients. In PD-L1 positive tumors, muscle invasion was seen in 48.1% (13 out of 27) of patients as compared to 18.2%(2 out of 11) of PD-L1 negative patients but it was not statistically significant. PD-L1 showed a higher expression in high grade tumors which was statistically significant (p=0.037).

Figure 12 : Urothelial carcinoma : Relationship between PD-L1 expression and clinicopathological factors



DISCUSSION

DISCUSSION

PD-1 and PD-L1 are promising targets for immunotherapeutic approaches, and they are considered novel markers with potential prognostic value (85). The expression of PD-L1 is currently being investigated as an important prognostic and predictive biomarker(86); however, it is still not validated alone to determine which patients should receive PD-1/L1 blockade therapy(87). The current study aimed at identifying PD-L1 expression on tumor cells in patients diagnosed with RCC, prostate carcinoma and urothelial carcinoma and correlate this to tumor characteristics and prognosis.

Prostate Carcinoma

In our study, the positive rate of PD-L1 expression in prostate cancer was 41.4% which was higher in patients >70 years age group, ECOG performance status 0-2, gleason score >7, with perineural invasion present and lymphovascular invasion absent.

In previous studies, a significant association of PD-L1 expression with adverse clinicopathological characteristics like higher PSA levels in prostate cancer was identified. For example, Gevensleben et al. revealed that clinicopathological features including proliferation, Gleason score and androgen receptor (AR) expression showed a positive association with moderate to high PD- L1 expression levels(88). Meanwhile, in 130 untreated African American ethnicity prostate cancers, Calagua et al. revealed that PD-L1 positivity was prognostic for biochemical recurrence.

Furthermore, the elevated serum PSA and small prostate independently predicted tumour PD-L1 positivity(89), whereas other reports showed different results and no significant association between PD-1/PD-L1expression and patient characteristics including the Gleason score, PSA, clinical TNM stage and pathological stage(90).

Renal Cell Carcinoma

PD-L1 positivity on tumor cells was recorded for 60% of the RCC patients. Studies that investigated PD-L1 expression by IHC have reported positivity rates ranging from 5 to 57% for tumor cells(91,92). In a study of 306 patients, PD-L1 positive expression was seen in 23% of cases(59). Additionally, in another study of 346 RCC patients, PD-L1 positivity in tumor cells was found in 14.9% of patients.. This great variation may be related to differences in

PD-L1 expression between RCC subtypes. The existing data regarding this subject are conflicting. A recent study reported lower rates of PD-L1 expression in clear cell compared to papillary (0–16% versus 27–32%) or in chromophobe RCC (0% versus 35%)(93). Similarly, in a meta-analysis, a significant difference in expression between clear cell and non-clear cell histology was detected(94). However, there are also studies showing higher PD- L1 positivity rates in clear cell RCCs than in other renal tumor subtypes(95,96). In our study, out of the patients with positive PD-L1expression, 55.6% was clear cell carcinoma and 44.4% was non clear cell carcinoma. The difference was non statistically significant. The small population and heterogeneity in histological subtypes may have affected our results. diagnosis and PD-L1 testing were conducted on the tissue of the primary tumor.

The type of technique used for the assessment of PD-L1 expression is still not standardized. Different techniques are utilized in different studies. In a recent meta-analysis, several studies conducted IHC on tumor tissue while others used ELISA in the serum of affected patients. When the analysis was limited to studies utilizing IHC, a marked difference in the risk of death related to the increased expression of PD-L1 was seen (risk of death 2 compared to 1.81)(94). All these factors along with the small sample size may have contributed to the difficulty in interpretation of our results regarding PD-L1 expression and thus rendering the comparison with other existing data inaccurate.

When correlating the PD-L1 tumor expression with clinical and pathological factors, we could not detect a statistically significant difference. Patients with PDL1 positive tumor cells had higher clear cell as histology (P=0.471), lower nuclear grade (P=0.987; yet all with no statistical significance. Several studies reported that in ccRCC, expression of PD-L1 is strongly correlated with aggressive features and prognosis(55,94,97). In a study that included patients with pRCC, no significant association was found between PD-L1 expression and all clinicopathological factors(98). Moreover, in a cohort of 81 chRCC patients, PD-L1 positivity was not associated with tumor aggressiveness. It was suggested that neither PD-1 positivity in inflammatory cells nor PD-L1 positivity in the tumor had an impact on the natural course of a chRCC tumor(99). Furthermore in another study, PD-L1 status was associated with parameters of aggressiveness but was not proven to be a significant independent prognostic biomarker(97).

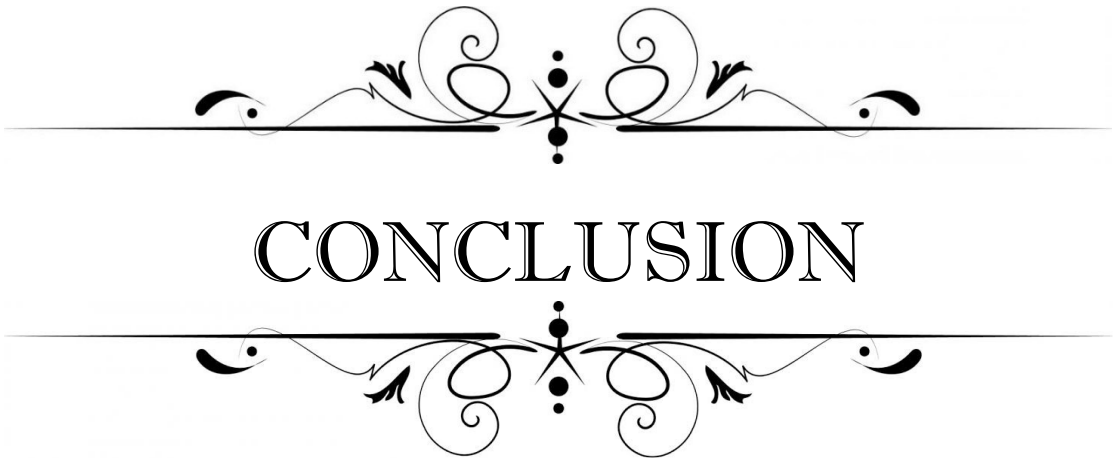
Urothelial Carcinoma

Although five immunotherapeutic agents (PD-1/PD-L1 inhibitors) have been approved by the US-FDA for use in bladder tumors, the appropriate assay for patient selection has remained controversial to date. Immunohistochemistry for PD-L1 and PD-1 is the most commonly used assay; however, the guidelines for positivity and clones to be used remain vague.

PD-L1 immunohistochemistry in the present study showed positivity in 71.05% of cases with a high expression in high- grade carcinomas. A high expression in high-grade and muscle-invasive carcinomas has also been shown in the previous study by Kawahara et al(100). Although previous studies have shown a slightly higher expression, this may be due to different clones used, a higher number of invasive and high-grade carcinomas, tumor heterogeneity, and the different cut-offs for positivity(101,102).

Differences in age, gender did not show any statistically significant difference in the expression of either PD-L1. A previous study by Holland et al correlating clinicopathological features with PD-1 and PD-L1 expression has also shown no impact of age and sex on the expression(103).

Recently, the US FDA has approved the use of Atezolizumab and Pembrolizumab for first-line use in platinum therapy-ineligible patients only in PD-L1 positive tumors(104). This has opened up the scope of compulsory PD-L1 testing in patients with urothelial carcinoma ineligible for platinum-based therapies; however, the availability of companion diagnostic approved is scarce and laboratory dependent. There is a marked demand for the development and validation of laboratory-dependent tests so that these may be used before treatment. This will promote the use of immunotherapeutic agents on a larger scale even in resource-poor settings.



CONCLUSION

CONCLUSION

In the available literature, there has been great discordance in PD-L1 expression and relationship with various clinicopathologic features. Our study is the one with the large sample size conducted on the Indian population of various genitourinary malignancies, and it revealed similar as well as different results in various aspects from other similar studies of the past. There were many differences among PD-L1 expression in GU malignancies but in urothelial carcinoma, high grade tumors had statistically significant higher PD-L1 expression as compared to low grade tumors. Such great variation shows that further studies are required on a larger scale from different geographic areas to have an actual understanding of biological behavior of PD-L1 and to answer the question of judicious implication of therapeutic agents targeting these receptors for benefitting patients who do not respond to or progress on conventional therapeutic modalities.

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ANNEXURES

ANNEXURES

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2021/3505 **Date: 12/03/2021**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3340**

Project title: **"Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**M.Ch. Dissertation**
Student Name:	**Dr. Amit Aggarwal**
Guide:	**Dr. Vijay Kumar Sarma Madduri**
Co-Guide:	**Dr. Gautam Ram Choudhary, Dr. Himanshu Pandey, Dr. Mahendra Singh, Dr. Arvind Sinha & Dr. Meenakshi Rao**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary

Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Basni Phase-2, Jodhpur, Rajasthan-342005; **Website:** www.aiimsjodhpur.edu.in; **Phone:** 0291-2740741 Extn. 3109
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अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
अनुसंधान अनुभाग
Research Section

No.: AIIMS/RES/2021/6681 Dated: 3/11/21

To
Dr. Vijay Kumar Sarma Madduri
Assistant Professor,
Department of Urology,
AIIMS, Jodhpur.

Subject: Handling over of PG Thesis: Reg.

Dear Dr. Kumar,

This is in reference to your letter no. AIIMS/JDH/2021/URO-224 dated 23/10/2021. I am directed to inform you that Dean (Research) accorded his permission to change the guide of following students to respective co-guide as per your request, if they are eligible for guideship as per institutional guidelines. Details as follows:

Sr. No.	Name of Student	Session	Thesis Title	Changed to
1.	Dr. Shakti Swaroop Sarangi	July-2020	Comparison of Radial Artery Deviation and Reimplantation (RADAR) Technique vs Classical Technique in Creation of Arterio-venous Fistula: A Randomised Control Trial	Dr. A S Sandhu
2.	Dr. Amit Aggarwal	July-2020	Programmed Death-Ligand 1 (PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features.	Dr. Gautam Ram Choudhary

Dr. Jaykaran Charan
Sub Dean (Research)

Sub Dean (Research)
All India Institute of Medical Sciences
Jodhpur (Raj.)-342005 India

Copy for Information to: -
1. Dr. A S Sandhu, Professor & Head, Dept. of Urology, AIIMS, Jodhpur.
2. Dr. Gautam Ram Choudhary, Associate Professor, Dept. of Urology, AIIMS, Jodhpur.
3. Concern PG Student.
4. Member Secretary, IEC, AIIMS. Jodhpur.

Basni Phase-2, Jodhpur, Rajasthan-342005, **Website:** www.aiimsjodhpur.edu.in, **Phone:** 0291-2740741 Extn. 3109
Email: deanresearch@aiimsjodhpur.edu.in, reserachcell@aiimsjodhpur.edu.in

PROFORMA

Name:

Age: Sex:

Patient's ID:

Address with phone number:

Addiction:

Comorbidity:

Chief complaints and duration:

Previous surgical history:

Personal history:

Family history:

Treatment history:

Any known allergy:

General and systemic examination:

Laboratory parameters:

1) complete blood count (CBC)
    a. Hemoglobin
    b. Total leucocyte count
    c. Differential leucocyte count
    d. Platelets
2) kidney function test (KFT)
    a. Blood urea
    b. Serum creatinine
3) serum electrolytes (SE)
    a. Sodium
    b. Potassium
4) liver function test (LFT)
    a. Alanine transaminase(ALT)
    b. Aspartate transaminase (AST)
    c. Serum alkaline phosphatase (ALP)
    d. Total bilirubin
    e. Direct bilirubin
    f. Indirect bilirubin
    g. Total protein
    h. Albumin

i. Globulin

Date of admission:

Date of surgery/ biopsy:

Radiological findings:

USG KUB:

CT Urography:

MR urography:

Surgery details:

Histopathologic report:

Tumor type

Tumor stage

- Radiological
- Pathological (if available)

Tumor grade

Immunohistochemical assay

PD-L1

ANNEXURES

CONSENT FORM

I_____S/O, W/O, D/O_____

R/O_____,

Exercising my free power of choice, hereby give my consent to be included as a subject in the study entitled : "Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features".

I have been given a full explanation by the study doctor of the nature, purpose and the likely duration of the study and what I will be expected to do and I have been advised any foreseeable risk associated with the procedure. This has been explained to me in the language I best understand.

I agree to cooperate fully with the supervising doctor and to inform him/her immediately if I suffer from any unusual symptoms during the study period. I am also aware of my right to opt out at any stage of the trial during the course of study and my usual treatment will be continue. I understand that medical records that reveal my identity will be kept confidential.

Name of the patient:

Signature:

Name of supervising doctor

Dr. Amit Aggarwal

Guide:

Dr. Gautam Ram Choudhary

Additional Professor

Name of accompanying relative:

Signature:

सहमतिपत्र

मैं_____

पत्नी/बेटी/पति/बेटा_____निवासी_____

_____

अपनी इच्छा अनुसार "Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features"

नामकशोध अध्ययन में एक अध्ययन विषय के रूप में शामिल होने के लिए अपनी पूर्ण स्वतंत्र और स्वैच्छिक सहमति देता / देती हूँ । मुझे पूर्ण संतुष्टि अनुसार ड्यूटी पे तैनात चिकित्सक द्वारा अध्ययन के उद्देश्य के बारे में स्पष्ट स्पष्ट बता दिया गया हैँ। मुझे बता दिया गया है की इस शोधकार्य में मेरी भागीदारी पूरी तरह स्वैछिक है और मैं किसी भी दण्ड के भय के बिना और ऐसा करने के मेरे कारण को दिए बिना किसी भी समय इस अध्ययन से अपनी भागीदारी समाप्त करने के लिए चुन सकता / सकती हूँ। मैं इस शोध कार्य में भाग लेने के लिए किसी भी मौद्रिकयावित्तीय या मुआवज़े के किसी अन्यरूप का दावा नहीं करूंगा / करुंगी । मैं समझती हूँ की मेरी बिमारी की जानकारी एवं उस सेसम्बंधित सभी वस्तुएं गोपनीय रखी जाएँगी लेकिन रोगी की गोपनीयता अनुसंधानकाविशेय है और अन्य स्वास्थ्य सेवा प्रदाताओं की रक्षा हेतु इसे तोड़ा जा सकता है।

मैं अपने हस्ताक्षर द्वारा इसशोध कार्य मैं पूरा सहयोग एवं बिमारी से सम्बंधित जानकारी डॉक्टर के साथ बाटने का वादा करता / करती हूँ।

मरीज़ का नाम: मरीज़ के हस्ताक्षर

तिथि :

छात्र: डॉ. अमित अग्रवाल

पर्यवेक्षक: डॉ. गौतम राम चौधरी

अतिरिक्त प्रोफेसर

यूरोलॉजी विभाग, एम्स जोधपुर

गवाह 1 का नाम : गवाह 1 के हस्ताक्षर

PATIENT INFORMATION FORM

Patient Identification Number for this research project:

Title: "Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features"

Student : Dr. AMIT AGGARWAL

Supervisor : Dr. GAUTAM RAM CHOUDHARY
Additional Professor
Department of Urology, AIIMS Jodhpur

You are being invited to participate in this research to assess: "Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features"

PURPOSE OF RESEARCH: "Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features"

EXPECTED DURATION OF PARTICIPATION: Patient's follow up will be divided in to: 1) short duration follow up (due to restricted study duration) and 2) long duration follow up till death of patient or lost to follow up.

FORESEEABLE RISKS BY PARTICIPATING IN THE STUDY: Patient will undergo normal treatment protocol, investigations and surgeries risks will be involved but there is no extra risk due to participation in this study.

BENEFITS BY PARTICIPATING IN THE STUDY: No direct benefit would be there as far as the present study is concerned. However, it may beneficial to the society and the other people if this study will able to help in modifying the management of patients.

ALTERNATIVES TO PARTICIPATION: None, as this is an observational study.

CONFIDENTIALITY: All the information that you or your patient provides during the study will be kept confidential on a password protected computer. Information collected about the patient from his/her participation in this research and sections of any of his/her medical notes will not be used for any other purpose but it may be looked at by responsible individuals.

COST OF PARTICIPATION: No additional cost to you for participating in this study.

PAYMENT FOR PARTICIPATION: No incentives to you for participating in this study.

In the event that at any time during the course of the study you/your patient feels that you/they have not been adequately informed as to the possible risks, benefits, alternative procedures, or rights as a study subject or feel under pressure to continue against your wish you can contact:

Principal investigator:

Dr. Amit Aggarwal
Department of Urology,
AIIMS Jodhpur, 342005
Tel: 8791255753

Dr. Gautam Ram Choudhary
Additional Professor,
Department of Urology,
AIIMS Jodhpur, 342005

LEGAL RIGHTS: By signing this form, we are not violating any of your legal rights. The patient or patient's relative will be notified in a timely manner if significant new findings develop during the course of the research which may affect the subject's willingness to continue participation.

Date:

Place:

Signature/left thumb impression of the patient: _____

Patient's name:_____

Signature of principal investigator:
Date:

रोगी सूचना पत्र

इस शोध परियोजना के लिए रोगी पहचान संख्या:

शीर्षक: "Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features"

छात्र: डॉ. अमित अग्रवाल

पर्यवेक्षक: डॉ. गौतम राम चौधरी

अतिरिक्त प्रोफेसर

यूरोलॉजी विभाग, एम्स जोधपुर

मूल्यांकन के लिए आपको इस शोध में भाग लेने के लिए आमंत्रित किया जा रहा है:

"Programmed Death-Ligand 1(PD-L1) Expression in Genitourinary Malignancies and Correlation with Clinicopathologic Features"

भागीदारी की अपेक्षित अवधि: रोगी का अनुवर्ती भाग इस प्रकार विभाजित किया जाएगा:

1) छोटी अवधि अनुवर्ती (प्रतिबंधित अध्ययन अवधि के कारण) और

2) लंबे समय तक रोगी की मौत तक फॉलोअप या फॉलोअप के लिए खो दिया।

अध्ययन में भाग लेने के लिए जोखिम : आपके इलाज में होने वाले जोखिम से इस जांच का कोई नाता नहीं है. इसमें किसी भी प्रकार का अलग से जोखिम नहीं है .

अध्ययन में भाग लेने से लाभ: वर्तमान अध्ययन के संबंध में कोई प्रत्यक्ष लाभ नहीं होगा । हालांकि, यह समाज और अन्य लोगों के लिए फायदे मंद हो सकता है य दियi ह अध्ययन रोगियों के प्रबंधनमें संशोधन करने में मदद करेगा।

समूह आवंटन: कोई नहीं

साझेदारी के लिए विकल्प: कोई नहीं, क्योंकि यह एक अवलोकन अध्ययन है।

गोपनीयता: अध्ययन के दौरान आप या आप के रोगी द्वारा प्रदान की जाने वाली सभी जानकारी को पास वर्ड संरक्षित कंप्यूटर पर गोपनीय रखा जाएगा ।इस शोध में रोगी के बारे में एकत्रित जानकारी और उसके किसी भी चिकित्सा नोट के अनुभागों का उपयोग किसी भी अन्य उद्देश्य के लिए नहीं किया जाएगा, लेकिन इसे जिम्मेदार व्यक्तियों द्वारा देखा जा सकता है।

साझेदारी की लागत: इस अध्ययन में भाग लेने के लिए आपके लिए कोई अतिरिक्त लागत नहीं है।

भागीदारी के लिए भुगतान: इस अध्ययन में भाग लेने के लिए आपके लिए कोई प्रोत्साहन नहीं।

यदि किसी भी समय अध्ययन के दौरान आप / आप के रोगी को लगता है कि आपको संभावित जोखिम, लाभ, वैकल्पिक प्रक्रियाओ या अध्ययन विषय के रूप में अधिकारों के रूप में पर्याप्त रूप से सूचित नहीं किया गया है या जारी रखने के दबाव में महसूस नही किया गया है आपकी इच्छा के विरुद्ध आप संपर्क कर सकतेहैं।

मुख्य जांचकर्ता का नाम:

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यूरोलॉजी विभाग,
एम्स जोधपुर

कानूनी अधिकार :इस फॉर्म पर हस्ताक्षर करके, हम आपके किसी भी कानूनी अधिकार का उल्लंघन नहीं कर रहे हैं।

रोगी या रोगी के रिश्तेदार को समय-समय पर अधिसूचित किया जाएगा यदि अनुसंधान के दौरान महत्वपूर्ण नए निष्कर्ष विकसित होते हैं जो कि विषय जारी रखने की इच्छा को प्रभावित कर सकते हैं।

दिनांक:

जगह:

रोगी के हस्ताक्षर / बाएं अंगूठे की छाप: _____

मरीज का नाम:_____

प्रिंसिपल अन्वेषक का हस्ताक्षर:

दिनांक:

सह-जांचकर्ता का हस्ताक्षर:

दिनांक: