their biological behavior has not fully been elucidated, and the management has not been optimized. In the present study, we analyzed non-invasive recurrences after bladder-preserving therapy for invasive bladder cancer.

METHODS: One hundred patients with muscle-invasive bladder cancer (T2-3N0M0) underwent bladder-preserving therapy consisted of intra-arterial chemotherapy with methotrexate and cisplatin and radiotherapy. Among them, bladder was preserved and the treatment was completed in 81 cases. After a median follow-up period of 36 months, 22 patients (27%) experienced intravesical recurrence. Non-invasive recurrence developed in 18 cases and invasive recurrence in 3. One of the patients with non-invasive recurrence simultaneously experienced lung metastasis. In the present study, analysis was performed on the 17 patients with non-invasive recurrence alone.

RESULTS: The median age of the patients at presentation was 64 years old. Thirteen patients were male and 4 were female. T stages of the original tumors were T2 in 11 cases and T3 in 6 cases. Histological grades were G2 in 2 case and G3 in 15 cases.

Non-invasive recurrences developed at a median of 14 months after bladder-preserving therapy. T stages of the recurrent tumors were Tis in 5 cases, Ta in 6 cases and T1 in 6 cases. Histological grades were G2 in 10 cases and G3 in 2 cases. Treatments of the non-invasive recurrences were TURBT alone in 5 cases, TURBT followed by intra-vesical instillation of Bacillus Calmette-Guerin in 8 cases and cystectomy in 4 cases. Two of 10 cases who were treated by TURBT experienced an additional non-invasive recurrence. Finally, none died of bladder cancer and 2 patients died of other causes. Overall survival rate of the patients with non-invasive recurrence was not different from that of 54 patients without any recurrence (92.3% and 92.1% at 5 years, respectively).

CONCLUSIONS: Most of the recurrences after bladder-preserving therapy were non-invasive bladder cancer. Recurrent tumors were less aggressive than the original ones, and could effectively be managed by conservative treatment.

Source of Funding: None

490 PERIOPERATIVE OUTCOMES OF LAPAROSCOPIC RADICAL NEPHROURETERECTOMY AND REGIONAL LYMPHADENECTOMY IN PATIENTS WITH UPPER URINARY TRACT UROTHELIAL CARCINOMA AFTER NEOADJUVANT CHEMOTHERAPY


INTRODUCTION AND OBJECTIVES: To determine the effect of neoadjuvant chemotherapy (NAC) on surgical outcomes in patients undergoing laparoscopic radical nephroureterectomy (LNUX) for upper urinary tract urothelial carcinoma (UTUC).

METHODS: We performed a retrospective review of all UTUC patients who underwent LNUX performed at our institution between 1/2003 and 6/2010. Patients with high-grade tumor on biopsy and large tumor volume, sessile tumor, or status post cystectomy were considered high-risk and recommended to undergo NAC. High risk patients also underwent lymphadenectomy whether or not chemotherapy was given. A standard laparoscopic approach was undertaken in all cases with a Gibson incision used for dissection of the distal ureter and bladder cuff and intact specimen extraction. We compared differences in demographic, clinicopathological, and operative parameters between patients who underwent LNUX after NAC and patients who underwent initial LNUX. Logistic regression analysis was performed to identify predictors of complications.

RESULTS: We identified 82 UTUC patients who underwent LNUX; 26 received NAC. Patients who underwent LNUX after NAC had a higher body mass index, higher biopsy tumor grade (as expected based on selection criteria), and longer operative time than patients who underwent LNUX without NAC. Patients who received NAC underwent regional lymphadenectomy more often, with more lymph nodes (median number of nodes removed 7.5 vs 4, p = 0.009) and lymphoadipose tissue removed (median 34.1 cm³ vs 7.7, p = 0.002), than patients who did not receive NAC. NAC resulted in a 15% complete remission rate. No differences in median estimated blood loss, intraoperative transfusions, and length of hospitalization between the two groups were found. Perioperative complication rates were similar in both groups.

CONCLUSIONS: Aside from a longer operative time likely as a result of a regional lymphadenectomy, we found no differences in surgical outcomes between patients who underwent LNUX after NAC and patients who underwent LNUX without NAC. The neoadjuvant approach resulted in a 15% rate of complete remission, similar to recently published data on UTUC that also reported a 43% reduction in stage T3–T4 disease. Our findings support the use of LNUX in selected patients undergoing neoadjuvant chemotherapy for UTUC.

Source of Funding: This study was supported in part by the National Institutes of Health Grant CA091846-08 and Cancer Center Support Grant CA 016672.

491 PUTATIVE CANCER STEM CELLS (CSCS) SIGNALING AFTER IMMUNOTHERAPY WITH BACILLUS CALMETTE-GUERIN (BCG) AND P-MAPA IN THE SUPERFICIAL BLADDER CANCER (SBC)

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INTRODUCTION AND OBJECTIVES: High recurrence rate and heterogeneous presentation of SBC prompted us to suppose that stem cells could be involved. Intravesical BCG is considered the most successful treatment for SBC. The extracellular purified compound isolated from Aspergillus oryzae was characterized as an aggregated polymeric form of protein magnesium ammonium phosphonolipate-palmitoleate anhydride (P-MAPA), having significant in vivo antitumor and antibacterial activities and no toxicity. The aims of this work were to characterize the putative CSCs features in the SBC of rats submitted to intravesical immunotherapy, and to establish the response of these cells to BCG and P-MAPA.

METHODS: Thirty female Fisher 344 (7 week old) rats were anesthetized and received 1.5 mg/kg dose of n-methyl-n-nitrosourea (MNU), intravesically every other week for 7 weeks. After MNU treatment, the 30 rats were divided into 3 groups: The MNU group received 0.30 ml dose of 0.9% physiological saline; The BCG group received 10⁶ UFC dose of BCG; The P-MAPA group received 5 mg/kg dose of P-MAPA, all intravesically for 8 weeks. After 15 weeks, all bladders were collected for immunological and Western Blotting analysis for CD44, CD133, CD117, CK5, ATP-binding cassette membrane transporters (ABC2G2), p53, Toll-Like Receptor (TLR) 2 and 4, Ki-67 (cellular proliferation) and apoptosis detection.

RESULTS: CSCs were ABC2G2/CD44/CD133+, which were more often in the MNU group and decreased in the BCG and P-MAPA groups. Also, normal urothelial stem cells (USCs) CD44/CD133/ CD117/p53/CK5+ were mainly increased in the P-MAPA group. The p53 immunoreactivity was 5% staining of urothelial cells in the MNU group, 20% in the BCG group and > 50% in the P-MAPA group. The TLR 2 and 4 immunoreactivities were 2% staining of urothelial cells in the MNU group, 11% in the BCG group and > 50% in the P-MAPA group. The apoptosis and cellular proliferation indexes were increased in all experimental groups. However, these processes were decreased in the BCG and P-MAPA groups in relation to the MNU group (p<0.05).

CONCLUSIONS: BCG and P-MAPA showed involvement in the regulation of the p53, TLRs, apoptosis and cellular proliferation balance. However, P-MAPA was more sensitive in restoring p53 and TLRs immunoreactivities and balance between normal USCs and CSCs. P-MAPA therapy showed stimulatory effect on TLRs and p53
that was parallel to the decreased of CSCs. It could be concluded that the P-MAPA showed to be superior to BCG in the regulation of the CSCs focusing on TLRs and p53 homeostasis.

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**492**

**RECOMBINANT B7-DCIG ENHANCES BACILLUS CALMETTE-GURIN (BCG)-INDUCED TH1 AND ANTI-BLADDER CANCER IMMUNE RESPONSES IN VITRO AND IN VIVO**

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**INTRODUCTION AND OBJECTIVES:** B7-DClg, AMP-224, is a recombinant Fc fusion protein composed of the extracellular domain of B7-DC fused to the hinge and Fc domain of IgG. B7-DClg interacts with PD-1, reduces the dysfunctional CD8+ T cells, and promotes an effective antitumor response. In vitro *M. tuberculosis* stimulation of human PBMC upregulates PD-1 expression in T cells. We proposed to test if B7-DClg could enhance BCG-induced anti-bladder cancer immune responses.

**METHODS:** For in vitro evaluation of B7-DClg on BCG induction of immune responses, mouse splenocytes were prepared and cultured in the absence or presence of Paster strain BCG and/or different doses of B7-DClg for 3 days, followed by ELISA analysis of interferon gamma (IFN-γ) in culture supernatants. For in vivo evaluation of B7-DClg on BCG induction of immune responses, mice were treated twice weekly with intravesical (i.b.) PBS plus intraperitoneal (i.p.) BCG (0.1 OD/dose) plus i.p. IgG2a (400 μg/dose), or i.b. BCG (0.1 OD/dose) plus i.p. B7-DClg (400 μg/dose). The bladders were collected after treatments 2, 4, and 6, and analyzed for IFN-γ as well as other mRNAs by RT-PCR. ImageJ software was used to quantify PCR products on agarose gels. To evaluate the effect of B7-DClg on BCG treatment of bladder cancer, C57BL/6 mice were implanted i.b. with luciferase-expressing MB49 (MB49-Luc) bladder cancer cells at day 0 and treated with i.b. BCG (0.1 OD/dose) plus i.p. B7-DClg (400 μg/dose) twice weekly starting at day 1 for a total of 6 treatments. Control mice were treated with i.b. PBS plus i.p. PBS or i.b. BCG plus i.p. IgG2a. Bioluminescence was measured with Xenogen IVIS imaging at days 7, 14, and 21. At day 23 mice were euthanized and bladder weights measured.

**RESULTS:** B7-DClg enhanced BCG-induced splenocyte IFN-γ production in a dose-dependent manner in vitro. B7-DClg also enhanced BCG-induced bladder expression of IFN-γ and tumor necrosis factor related apoptosis-inducing ligand (TRAIL) mRNAs in vivo. In the orthotopic tumor model, BCG treatment reduced bladder weight by 53% (p = 0.0503 vs. PBS-treated group). Combination of BCG with B7-DClg further reduced bladder weight by 5% (p = 0.0257 vs. PBS-treated group). In addition, the combination treatment also led to a delay in tumor growth by bioluminescence compared to BCG-treated group.

**CONCLUSIONS:** B7-DClg enhanced BCG-induced Th1 and anti-bladder cancer immune responses in mice. B7-DClg may serve as a potential agent in combination with BCG for the treatment of bladder cancer in humans.

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**V493**

**RETROPERITONEAL LAPARO-ENDOSCOPIC SINGLE SITE (LESS) DONOR NEPHRECTOMY - DESCRIPTION OF A NEW TECHNIQUE**

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**INTRODUCTION AND OBJECTIVES:** Donor nephrectomy with laparo-endoscopic single site (LESS) surgery has been reported via the transperitoneal approach. The umbilicus (e-NOTES) is a popular site, but leaves a nasty scar and extraction of the kidney may be complex risking warm ischemic renal injury. Our units have a combined experience of around 300 retroperitoneal donor nephrectomies and therefore want to offer single site surgery to our patients.

**METHODS:** We describe a novel technique of LESS retroperitoneal donor nephrectomy using a single surgical incision in the groin, below the abdominal skin crease or “bikini line” using a GelportTM and bariatric/curved laparoscopic instruments. The LESS groin incision offers superior cosmesis, while the retroperitoneal approach has distinct advantages, such as the ability to identify the renal vessels early and not violating the peritoneal cavity. The new procedure has been performed in two patients on the right side (body mass index 32 and 33 kg/m2, respectively).

**RESULTS:** The operative times were 4 and 5 hours, warm ischemic times 135 and 315 seconds, blood loss 100 and 250 ml, and hospitalization 3 and 2 days, respectively. Graft function was good during the early post-operative period.

**CONCLUSIONS:** Retroperitoneal LESS donor nephrectomy through a single, inconspicuous groin incision is feasible and safe. Further evaluation of the technique in a larger patient cohort is indicated and underway.

**Source of Funding:** None

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**V494**

**ROBOTIC LIVE DONOR NEPHRECTOMY: FIRST EXPERIENCE AT A TERTIARY CENTER**

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**INTRODUCTION AND OBJECTIVES:** We herein describe our initial experience with an entirely robotic-assisted laparoscopic live donor nephrectomy (RALDN). As far as our knowledge this represents also the first experience in Spain.

**METHODS:** A 45 year old man was found to be a perfect match to his 44 year old spouse, who was affected by renal insufficiency due to diabetes and high blood pressure. The steps of the robot assisted live donor nephrectomy are herein described and include: 1. Mobilization of the colon down to the level of the iliac vessels 2. Complete mobilization of the spleen to the crus of the diaphragm, 3. Dissection of the adrenal gland and mobilization of the upper pole of the kidney 4. Identification of the renal hilum and gonadal vein 5. Transsection of the gonadal vein 2.5 cm from the renal vein 6. Identification of the ureter and dissection between the gonadal vein and the ureter to the level of the common iliac artery 7. Elevation of the kidney and posterior dissection with identification of the renal artery 8. Dissection of the lateral attachment of the kidney 9. Pfannenstiel incision and insertion of a 15 mm trocar 10. Stapling of the renal artery, renal vein and ureter through the 15 mm trocar 11. Extraction of the specimen with a 15 mm endo-bag.

**RESULTS:** The procedure was entirely performed with the robotic assistance. The operative time was 145 min. The warm isch-