CTL/Treg ratio in the presence of PD-L1+ TIM (favorable). This indicates that PD-L1+ TIM and PD-L1 margin expression were independently predictive in absence of HPV, the interaction between CTL/Treg ratio and PD-L1+ TIM was predictive irrespectively of HPV-status.

CONCLUSIONS: A PD-L1 expression pattern predominantly at the tumor-stroma margin predicts good prognosis, while the negative predictive value of PD-L1+ TIM appear to be compensated by a high CTL/Treg ratio. All independent prognostic factors are PD-L1 related parameters. These results strengthen the rationale for anti-PD-1/PD-L1 immunotherapy in penile carcinoma.

Source of Funding: none.

MP44-19

HIGH-THROUGHPUT CHEMICAL SCREENING FOR SENSITIZATION OF BLADDER CANCER TO GEMCITABINE AND CISPLATIN CHEMOTHERAPY

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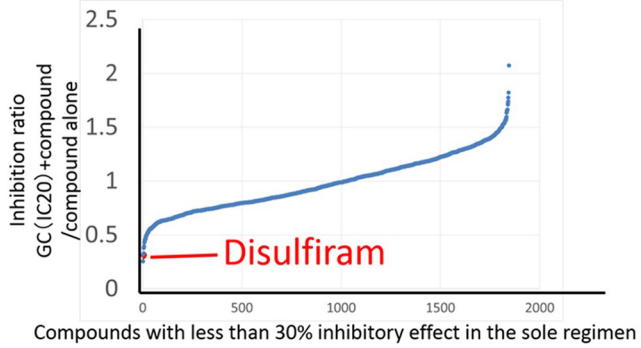
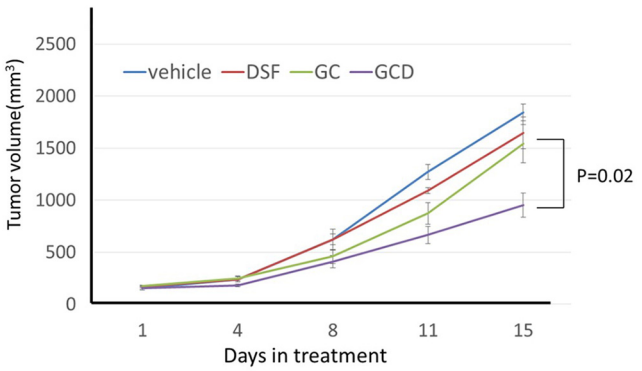
INTRODUCTION AND OBJECTIVES: Gemcitabine and cisplatin chemotherapy (GC) is the current standard regimen for locally advanced and metastatic bladder cancer (BC). Despite a relatively high initial response rate, some cases do not regress (intrinsic resistance) and the remaining cases often show regrowth after initial shrinkage (acquired resistance). To identify novel therapeutic agents for overcoming these resistances, we applied a high-throughput screening of chemicals administered in combination with GC.

METHODS: As a high-throughput screening, 2100 compounds were administered alone or in combination with GC to human BC cell lines (J82, UMUC-3). Cell viability was determined after 3-day incubation and chemicals that enhanced inhibitory effect of GC were screened. The in vivo effect of disulfiram (DSF) was studied in UMUC-3 cell xenografts, and western blot, immunofluorescence, induced coupled plasma spectrometry and measurement of reactive oxygen species (ROS) were done in vitro for mechanistic exploration.

RESULTS: The initial screening identified 26 compounds and further validation narrowed them into the most synergistic agent disulfiram, an FDA-approved drug for alcoholism. Combination index assay showed synergistic effects of DSF with cisplatin but not with gemcitabine in J82, UMUC-3, T24, HT1197 and HT1376 cells. Co-administration of DSF significantly increased DNA-platinum adducts by regulating cisplatin efflux transporter ATP7A and enhanced apoptosis by GC treatment in UMUC-3 cells, with significant increase of ROS production. Use of DSF in combination with GC (GCD) significantly inhibited tumor growth of UMUC-3 subcutaneous xenograft on athymic mice (by 39% compared with GC alone, $p = 0.02$). GCD regimen was as tolerable as GC and no significant differences were observed in body weight of treated mice between the two regimens.

CONCLUSIONS: Repositioning of DSF to a chemotherapy sensitizer is a promising treatment strategy, which can be translated rapidly in the future.





Source of Funding: none

MP44-20

EGFR CELL EXPRESSION IN BLADDER WASHINGS AS A RISK MARKER TOOL IN NON MUSCLE-INVASIVE BLADDER CANCER. PRELIMINARY EXPERIENCE

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INTRODUCTION AND OBJECTIVES: Up to day, EGFR expression has been determined mainly in tissue specimens of muscle-invasive bladder cancer and its overexpression has been associated with worse prognosis and shorter survival. Urothelial EGFR status after NMIBC transurethral resection (TUR) could indicate the risk of recurrence and progression. We investigated the feasibility of EGFR measurement in bladder washings of patients undergoing intravesical adjuvant therapy for NMIBC and its usefulness in identifying risk subgroups.

METHODS: Our prospective study included patients after TUR of NMIBC and healthy controls. A cellular pellet was obtained from bladder washing, and RNA extraction performed by miRNeasy Mini Kit (Qiagen®). Good quality of RNA was checked. The cDNA obtained from RNA was used to perform a gene expression analysis by a Real Time PCR, according to the method of the comparative quantification ($\Delta\Delta Ct$) with an endogenous control (Cyclophilin). Every reaction was set in triplicate as a guarantee of quality. Patients were grouped for EAU risk class and maintained in follow-up. The EGFR expressions were statistically analyzed according to EAU risk groups and to patients' outcome. EGFR gene expression values were expressed in FOLDs of change compared to healthy controls (EGFR=1).

RESULTS: Fifty-eight patients and 21 healthy age-matched controls were entered. An adequate cellular pellet was obtained in 50 patients (86.2%) showing a median EGFR expression of 2.0 folds (IQR 0.6-4.3, p=0.0004). After TUR and adjuvant intravesical therapy, 22 (55%) out of 40 high-risk patients, showed EGFR decrease to 1.3 folds (IQR 0.9-1.5), while 18 (45%) showed elevated EGFR, median 4.7 (IQR 4.1-11.6). At 25 months median follow-up (IQR 19.0-34.8), 20 (40%) patients recurred and 6 (12%) progressed. Among patients with or without EGFR gene increase, 9 (22.5%) and 5 (12.5%) recurred and 5 (12.5%) and 1 (2.5%) progressed, respectively.

CONCLUSIONS: In our experience EGFR expression measurement was feasible in more than 85% of patients and resulted related to EAU risk classes for recurrence and progression, showing different behavior during intravesical therapy. It was possible to identify a subgroup of high risk patients overexpressing EGFR in spite of intravesical adjuvant therapy. EGFR evaluation in bladder washing could represent a repeatable and useful tool to identify a subgroup of patients at risk for progression unresponsive to intravesical adjuvant therapy and candidate to early radical cystectomy.

Source of Funding: none