MONOGRAPHIC: TUMOR MARKERS IN UROLOGICAL CANCERS: AN UPDATE
Arch. Esp. Urol. 2022, 75 (2): 144-155

OPTIMIZATION OF THE DIFFERENT THERAPEUTIC STRATEGIES IN MUSCLE INVASIVE BLADDER CANCER USING BIOMARKERS

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Summary.- Predicting response to definitive treatments is a fascinating challenge which develops through the evolution of a panel of convincing molecular biomarkers capable of adding in clinical decissions despite interpatient and intratumoral heterogenicity. Muscle-invasive bladder cancer (MIBC) can be locally treated either with radical cystectomy (RC) with or without neoadyuvant chemotherapy or bladder preservation approaches such as trimodal therapy (TMT) including maximal transurethral resection of bladder tumor (TURBt) followed by external beam radiotherapy with concurrent systemic radio-sensitizing chemotherapy. Conventional or novel/targeted systemic agents are essential parts of perioperative multidisciplinary management considering both neoadjuvant and adjuvant setting. Advances in molecular biology such as next generation sequencing and whole genome or transcriptomic analysis, provided novel insights to achieve a full understanding of the biology behind MIBC helping to identify emerging predictive signatures. Although several progresses have been made, real-world application of molecular biomarkers in MIBC scenario is hindered by lack of standardization, and low reproducibility. In this review we aim to present the emerging role of novel molecular biomarkers in predicting response to local treatments and systemic agents in MIBC.

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Key words: *Bladder cancer. Biomarkers. Neoadjuvant chemotherapy. Adjuvant chemotherapy. Radical cystectomy. Radiation therapy.*

Resumen.- La predicción de respuesta a tratamientos definitivos en un tumor es un desafío fascinante mediada por un panel de biomarcadores que pudieran aportar información para tomar decisiones terapéuticas, pese a la heterogenicidad entre pacientes e intratumoral. El tumor vesical infiltrante (TVI) puede tratarse con cistectomía radical (CR) con o sin neoadyuvancia (NAD) o estrategias de preservación vesical como la terapia trimodal (TMT), que incluye resección transuretral (RTU) máxima seguida de radioterapia externa con una quimioterapia radiosensibilizadora concomitante. Las terapias convencionales o dirigidas son esenciales dentro del manejo multidisciplinar necesario en sus facetas neo y adyuvantes. Los avances en biología molecular, como la secuenciación moderna y el análisis transcriptómico

del genoma, han permitido empezar a entender el comportamiento molecular del TVI ayudando a identificar firmas predictivas. Aunque se han hecho progresos, la aplicación asistencial de biomarcadores en TVI está frenada por la falta de estandarización y la baja reproducibilidad de distintos resultados esperanzadores. En esta revisión, pretendemos exponer el papel emergente de nuevos marcadores moleculares en la predicción de respuesta a tratamientos locales y sistémicos en el TVI.

Palabras clave: *Tumor vesical. Biomarcadores. Quimioterapia neoadyuvante. Quimioterapia adyuvante. Cistectomía radical. Radioterapia adyuvante.*

INTRODUCTION

Urothelial carcinoma (UC), which encompasses bladder cancer (BC) and upper tract urothelial carcinoma (UTUC) represents the sixth most commonly diagnosed cancer in Western Countries(1,2). Focusing on BC, it can present as a very heterogeneous disease comprising both non-muscle-invasive (NMIBC) and muscle-invasive (MIBC) with different oncological outcomes. Particularly, MIBC could embrace different clinical scenario. Radical cystectomy (RC) with pelvic lymph node dissection (PLND) represents a mainstay in the treatment of MIBC providing both pelvic cancer control and survival(3). Neoadjuvant chemotherapy (NAC) represents an essential part in this surgical-based strategy as well as adjuvant chemotherapy (AC) in patients harboring postoperative high-risk features(3). Specifically, NAC has evolved as the standard of care for treatment with curative-intent and pathological complete response (pCR), pathologic downstaging (pDS) or clinical complete response (cCR) are strong predictors of survival(4,5).RC and urinary diversion (UD) is a complex surgical procedure with a recognized high perioperative morbidity due to patient, disease and surgical determinants(6). Thus, more emphasis has been placed in favor of bladder sparing treatments for patients who are unfit for or aim to avoid RC without impairing outcomes(7,8). Bladder preservation in the context of MIBC primarily refers to trimodal therapy (TMT) including maximal transurethral resection of bladder tumor (TURBt) followed by external beam radiotherapy with concurrent systemic radio-sensitizing chemotherapy(3).There is a relevant unmet need for reliable biomarkers to guide the optimal choice of therapy. Although several clinical and pathological tools have demonstrated acceptable reliability capable of influencing survival outcomes(9–14), currently no molecular biomarkers are used in the clinical daily practice. Nowadays medical decisions should be tailored to the individual patient based on predicted response to local treatment and systemic agents including conventional chemotherapy and novel immune checkpoint inhibitors (ICIs) or targeted agents in both neoadjuvant or adjuvant settings(15). Here, we summarize recent evidence about biomarkers-related signature that could be leveraged to guide therapeutic decisions, the optimal use of bladder preservation approaches, post-treatment monitoring, and predicting response to systemic agents in MIBC.

DNA ALTERATIONS IN MIBC

The MIBC genomic landscape is characterized by high tumor mutational burden (TMB), frequent chromosomal alterations, and recurrent mutations in known oncogenes and/or tumor suppressor genes. Mutations can accumulate via DNA damage process such as APOBEC-related mutagenesis or via loss of DNA Damage Response and Repair (DDR) genes pathways(16). Such instability is one of the leading causes of tumorigenesis and contributes to the expression of neoantigens that can activate CD8+ T-cell to act against tumors via cGAS/STING pathway(17).

DDR genes are regulators of double-helix repair following platin-based damage through processes like nucleotide excision repair (NER) pathway, mismatch repair proteins or homologous recombination(15). The presence of a putative deleterious DDR gene alterations in pretreatment tumor tissue strongly predicted increased vulnerability to cisplatin-based chemotherapy as well as to TMT(18). Excision Repair Cross Complementing 2 (ERCC2) is a core member of the NER pathway. Whole Exome Sequencing (WES) on pretreatment tumor and germline DNA of 50 patients who received different regimens of Cisplatin-based NAC showed that somatic ERCC2 mutations were significantly associated with pCR or pDS at time of RC(19). In a multicenter prospective phase II trial testing the efficacy and tolerability of six cycles of dose-dense Cisplatin Gemcitabine (ddCG) NAC, Iyer et al. performed a biomarker analysis using Next Generation Sequencing (NGS). ERCC2 mutations were identified as strongly predictive for chemosensitivity, pDS (< pT2) and better 2-years recurrence-free survival (RFS)(20). Based on NGS among 46 MIBC patients who received TMT Desai et al. found that deleterious DDR mutations, particularly recurrent alterations in ERCC2, were associated with improved oncological outcomes(21). Among two independent prospective datasets of MIBC patients who received neoadjuvant Methotrexate, Vinblastine sulfate, Doxorubicin hydrochloride (Adriamycin), and Cisplatin (MVAC) or ddCG, the 3-DDR-genes

signature including Ataxia Telangiectasia Mutated-1 (ATM), RB transcriptional corepressor-1 (RB1), and FA Complementation Group C (FANCC) predicted pathologic response and better progression-free survival (PFS) and overall survival (OS)(22). The RETAIN trial (NCT02710734) aims to evaluate a risk-adapted approach to help guide various bladder-sparing strategies including definitive chemotherapy, based on pathological response to neoadjuvant MVAC and DDR mutational profile. Patients with an alteration in ATM, RB1, FANCC or ERCC2 and no evidence of residual disease at restaging TURBt and imaging post-NAC will begin a predefined active surveillance regimen whereas their counterpart will receive a direct therapy. Recently, an interim analysis of RETAIN trial has been presented. The intention-to-treat analysis included seventy-seven patients enrolled at four academic centers. Among these, thirty-three (46%) had a mutation of interest and twenty-eight patients (39%) started an active surveillance programme. Of those, 14 (50%) experienced recurrence: two recurred with progressive disease and have died, five had MIBC with one eventual metastatic recurrence, and seven had NMIBC. Interestingly, 76% of those with a mutation were cT0 at post-NAC TURBt staging. Among all the patients, 50% had an RB1 mutation, for whom the recurrence rate was 62%, and 31% had an ERCC2 mutation, for whom the recurrence rate was 25%(23). Similarly, ALLIANCE (NCT03609216) is a phase II prospective trial that incorporates ddCG and bladder preservation for patients harboring DDR gene alterations. Primary endpoint is 3-year event-free survival defined as the proportion of patients without invasive or metastatic recurrence and who achieve < cT1 response to NAC(24).

Considering a biomarker analysis including a comprehensive genomic profiling and Programmed cell Death-Ligand 1 (PD-L1) combined positive score assessment (CPS, Dako 22C3 antibody) in the context of the PURE-01 study, Necchi et al. reported the complex interplay between high TMB and CPS with a linear association with pCR(25). Conversely, Bandini et al. found that TMB was not associated with pCR to Pembrolizumab on multivariable analysis(26). These conclusions were also supported by the ABACUS trial among 95 patients receiving neoadjuvant Atezolizumab(27). A partial agreement between ABACUS and PURE-01 trials is represented by the correlation with pCR and the level of pre-existing immunity, documented by CD8+ T-cell infiltration or by immune-related gene signatures. Biomarkers' analyses within the NABUCCO trial showed a trend toward a higher TMB in MIBC achieving pCR (pT0N0 or pTis/pTaN0) to Ipilimumab plus Nivolumab compared to non-responders. Furthermore, the pCR rate among PD-L1-positive MIBCs (CPS>10) was 73% compared to 33% among PD-L1-negative tumors(28).

Galsky et al. presented the first bladder-sparing trial (NCT03558087) in which unselected patients who achieved a cCR (normal cytology, imaging, and cT0/Ta) after four cycles of Nivolumab plus CG and refused RC were offered to receive adjuvant or maintenance Nivolumab up to four months. Among the 76 patients enrolled, a strong correlation between high TMB and mutated ERCC2 with cCR or pCR was demonstrated(29).

The controversial data about meiotic recombination 11 homolog (MRE11) required a mention among TMT candidates. Low nuclear/cytoplasmic MRE11 ratio staining has been shown to stratify survival outcomes among MIBC patients undergoing TMT within RTOG bladder-sparing trials(30). However, this association was not confirmed among patients enrolled in the recent BC2001 an BCON trials(31). Putative alterations in DDR genes appear to be useful to predict pathological response and even oncologic outcomes in MIBC patients treated with neoadjuvant systemic agents. However, neither TMB nor the activity of specific mutational signatures seems to provide mature data about their prognostic impact in the bladder-sparing setting.

TYROSINE KINASE RECEPTORS PATHWAY

Mutations in Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2)(32), Fibroblast Growth Factor Receptor 3 (FGFR3)(33,34) and Phosphatidylinositol 4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3Ca)(33) were found predictive in MIBC patients undergoing treatment with curative intent and were targetable with novel therapeutic agents. Yang et al. reported that ERBB2, FGFR3 and PIK3Ca were more commonly altered among patients who achieved pCR to neoadjuvant CG(33). Yuen Teo et al. retrospectively demonstrated that patients with FGFR3 mutations were associated with lower responses to Cisplatin-based NAC and had worse RFS(35). van Rhijn et al. analyzed FGFR3 mutations assessing their prognostic value in a cohort of 1000 chemotherapy-naive RC patients. Among FGFR3 mutant tumors, 73% had FGFR3 overexpression versus 22% among FGFR3 wild-type tumors. Oncogenic FGFR3 mutations were associated with favorable pathological features and good prognosis compared with patients with FGFR3 overexpressed tumors only. Potentially, FGFR3 mutant MIBCs would be likely to benefit from anti-FGFR3 therapy(34). In this context, targeted therapies have recently brought a paradigm change in the treatment of several malig-

nancies. Here, FGFR3 genetic alterations and related expression may constitute a potential candidate for such treatments. Infigratinib, a selective FGFR1-3 inhibitor, has shown promising clinical activity and tolerability in patients with advanced UC harboring FGFR3 alterations. PROOF 302 has been designed to investigate the efficacy and safety of Infigratinib versus placebo as adjuvant therapy in patients with high-risk features after RC and FGFR3 alterations. Ideally, MIBC with FGFR3 overexpression may represent a nonmutant group of tumors in which FGFR3 signaling suggests a potentially targetable pathway which might benefit from anti-FGFR3 treatment. Primary endpoint is represented by centrally reviewed disease-free survival (DFS). Exploratory endpoints include biomarkers' analyses such as circulating cell-free DNA (cfDNA) and RNA-mediated resistance mechanisms. Results are expected in 2024.

MOLECULAR SUBTYPES AND TRANSCRIPTIONAL BIOMARKERS

Tumor transcriptional profiling and molecular subtyping has defined key entities of UC characterized by distinct multigene signatures, varying expression of potential drug targets and differing response to local or systemic treatments(15). The Cancer Genome Atlas (TCGA) identified at least five different molecular categories for MIBC. Although a general agreement about division in luminal, basal/squamous and neuronal subtypes already exist, recently, an international panel of experts reached a consensus on a set of six molecular classes(36). Luminal papillary, luminal no specified, luminal unstable, stroma-rich, basal/squamous, and neuroendocrine-like have been defined as separate entities regarding underlying oncogenic signatures, immune-enriched features, histological and clinical characteristics, including outcomes. Basically, the basal-squamous subtype has the strongest immune expression signature and might benefit the most from conventional NAC. The luminal-papillary subtype is associated with targetable mutations (FGFR1-3) and better prognosis after surgical treatment with curative intent(24).

Choi et al. demonstrated that basal-like subtype was predictive for response to neoadjuvant CG, whereas the p53-like subtype poorly responded. Luminal papillary tumors harbored the best prognosis irrespective the treatment received(37). Whole transcriptome analysis suggests that luminal and basal tumors might have the best response rate among patients receiving Cisplatin-based NAC. Conversely, basal-subtypes correlated with worse OS among those who received upfront RC(38).

As newly recognized as an independent molecular subtype of conventional UC, neuroendocrine (NE)-like subtype was associated with comparable pDS rate but with worse cancer-specific survival (CSS) if compared to other entities(39). Commonly, NE-like subtype exhibited worse prognosis in most MIBC cohorts except for patients who received TMT(40).

Claudin-low tumors were characterized by the highest indication of immune-infiltration. Immune gene signatures included increased rates of RB1, E1A Binding Protein P300 (EP300), and Nuclear Receptor co-repressor 1 (NCOR1) mutations and decreased rates of FGFR3, E74 Like ETS Transcription Factor 3 (ELF3), and Lysine Demethylase 6A (KDM6A) mutations. Clinical relevance might be represented as an indicator of response to novel ICIs(41). In this context, among patients enrolled in the PURE-01 study, Necchi et al. showed that tumors with higher levels of pre-existing immune infiltration (Immune190) had a favorable clinical response to neoadjuvant Pembrolizumab. Specifically, basal subtypes with higher immune-signature showed 100% 2-year PFS after Pembrolizumab compared to their counterpart(42). Within the first pilot combination neoadjuvant trial (NCT02812420) with ant ctive for pCR or pDS. Higher expression of the four-gene signature including POU Class 2 Homeobox Associating Factor 1 (POU2AF1), Lysosome-associated membrane glycoprotein 3 (LAMP3), Cluster of differentiation 79A (CD79A) and Membrane Spanning 4-Domains A1 (MS4A1) was described among responders compared to non-responders(43). Activated immune-infiltrate signatures were further associated with improved disease-specific survival (DSS) after TMT but not in a separate cohort of patients treated with Cisplatin-based NAC and RC(44). All together these findings provide robust data for the evaluation of immune-infiltrate signatures as predictive biomarker in the context of clinical trials combining ICIs and TMT such as the INTACT (SWOG/NRG1806) trial.i-PD-L1 (Durvalumab) plus anti-CTLA-4 (Tremelimumab) in cisplatin-ineligible patients, harboring high-risk features, Gao et al. reported that an immune-enriched signature was predi.

LIQUID BIOPSY

Liquid biopsy, including circulating tumor cells (CTCs) or circulating cell-free/tumor DNA (cf/tDNA), represents a promising and minimally invasive technique that can be a useful tool to overcome the limits related to conventional diagnostic methods. Isolating and analyzing such materials from body fluids represents a crucial step to interrogate progressive

disease allowing longitudinal monitoring for BC recurrence and response to therapy(15).

CIRCULATING TUMOR CELLS (CTCS)

CTCs are rare cancer cells that have invaded the vasculature or lymphatics from a primary BC and have potential as a detection tool for risk stratification and longitudinal monitoring after treatment with curative intent. Numerous methods have been described to isolate these entities. Currently, the only FDA-cleared method for CTCs isolation is the CellSearch based on negative selection of white blood cell membrane markers such as CD45 and positive selection using magnetic beads coupled to anti-epithelial cell adhesion molecule (EpCAM) antibodies(15). Based on this approach, Soave et al. prospectively enrolled 185 MIBC patients treated with upfront RC potentially receiving Cisplatin-based AC. Presence of CTCs was significantly associated with worse survival outcomes in patients without AC administration. In patients who received AC, there was no difference in either endpoint between patients with or without presence of CTCs. Thus, CTCs may be useful for counseling and decision-making on administration of AC(45). Within a prospective cohort of 100 MIBC patients undergoing upfront RC and PLND, Rink et al. analyzed the clinical relevance and Human Epidermal Growth Factor Receptor 2 (HER2) expression on CTCs. The authors found that preoperative CTCs were already detectable in almost 25% of MIBC patients undergoing upfront surgery and were a powerful predictor of survival outcomes after adjusting for well-established clinicopathological features(46).

One study is evaluating the potential role of CTCs among MIBC patients undergoing TMT. Using negative-selection, microfluidic CTCs isolation approach coupled with sensitive digital droplet polymerase chain reaction (ddPCR) the authors developed and validated a CTC gene expression score (CTC-GES) to monitor response to TMT. Eight candidate genes including Peroxisome proliferator- activated receptor gamma (PPARG), Uroplakin 1A (UPK1A), Uroplakin 2 (UPK2), Keratin 14 (KRT14), Epidermal Growth Factor Receptor (EGFR), Cytokeratin 19 (KRT19), Transmembrane Protein 129 (TMEM129), and Desmoglein 2 (DSG2) were identified by differential expression analysis comparing RNA expression signatures data and were analytically validated and comprised the CTC-GES. This assay was able to predict a recurrent disease after TMT more than two months prior to radiological detection(47).

Two interventional trials are evaluating the role of CTCs in MIBC patients. The CirGuidance study (NTR4120) enrolled RC candidates with the hypothesis that patients without preoperative detectable CTCs have such a good prognosis, not justifying NAC. To this aim, CellSearch CTCs were evaluated in patients having cT2-T4aN0-N1M0 BC before treatment decision was made. Those without detectable CTCs were not allowed to receive NAC, while in patients in whom one or more CTCs were present, NAC was proposed. The selected cut-off of CTCs it's 1, with the primary endpoint of 2-years OS. Another interventional study named Treatment Of Metastatic Bladder Cancer at the Time Of Biochemical reLApse Following Radical Cystectomy (TOMBOLA) study (NCT04138628) was initiated. This study enrolls patients undergoing NAC and RC and monitors for the presence of cfDNA after RC using personalized ddPCR. If patients have detectable cfDNA and/or evidence of relapse on imaging, they receive adjuvant Atezolizumab assuming that early initiation of targeted therapy will result in improved survival. The primary endpoint is the number of patients reaching a complete response.

CIRCULATING CELL-FREE DNA (CFDNA) AND CIRCULATING TUMOR DNA (CTDNA)

While cfDNA in healthy persons is mainly derived from non-tumor cells, several studies have shown that cfDNA from patients with advanced cancer contains ctDNA. DNA can be released from tumor cells by different molecular processes such as cell apoptosis, immune response-related, necrosis, micrometastasis, and secretion(15). Thus, cfDNA comprises both tumor and non-tumor DNA in blood and analyzing the ctDNA fraction has been proposed as potential cancer biomarker(48).

In a comprehensive study of ctDNA in patients with BC, Christensen et al. addressed the prognostic and predictive value of ultra-deep sequencing of ctDNA in 68 patients who received Cisplatin-based (or Etoposide) NAC and RC for MIBC. A total of 656 plasma samples were procured at time of diagnosis, during NAC, before RC, and during surveillance. Quantitatively, the authors found that the presence of ctDNA before NAC was predictor of worse RFS and OS. Of note, after NAC and before RC in ctDNA-positive patients, a significantly higher overall 12-months recurrence rate was observed. No pCR was observed in ctDNA-positive patients and all pT0 patients were ctDNA-negative. After RC, ctDNA analysis correctly identified all patients who developed progressive disease during follow-up.

Qualitatively, expression profiling for tumor subtype and immune signature analyses found a high contribution of mutational signature associated with ERCC2 deleterious mutations in patients who responded to Cisplatin-based NAC(49).

Within the ABACUS trial, Powles et al. explored the role of ctDNA samples taken before and after neoadjuvant Atezolizumab in pre-RC setting. WES performed on tumor and matched normal samples identified 16 patient-specific clonal tumor mutations. These 16 mutations were used to design a bespoke multiplex PCR assay. The presence of two or more of the patient-specific clonal alterations in the plasma defined ctDNA positivity. Neoadjuvant Atezolizumab was associated with a reduction in ctDNA levels in patients who achieved a response (pDS or pCR). Non-responders did not show any marked alterations in ctDNA levels supporting a link between ctDNA dynamics and response to Atezolizumab(27).

Historically, it has been difficult to determine which patients have residual disease and which are cured after RC. Therefore, many patients who are cured by surgery are unnecessarily exposed to adjuvant treatments, and other patients with residual disease may not receive potentially beneficial adjuvant therapies until disease progression is detectable by classical imaging. In this context, IMvigor010 (NCT02450331) is a randomized adjuvant study comparing Atezolizumab to observation after RC for MIBC. ctDNA sampling and definition followed the same methodology reported above. In the biomarker-evaluable population, ctDNA-positive patients had improved DFS with adjuvant Atezolizumab compared to those undergoing observation. Whereas no difference in DFS was found between arms for ctDNA-negative patients. Furthermore, ctDNA-positive patients after RC showed worse OS, but had significantly improved OS with Atezolizumab administration compared with observation. Finally, among patients in which ctDNA was not detectable at six weeks after randomization within the Atezolizumab arm had improved OS compared with those who did not clear ctDNA(50). Together, these findings from IMvigor010 suggest that Atezolizumab represents an effective treatment among ctDNA-positive patients. Whereas no difference in survival outcomes between the Atezolizumab and the observation arms were reported in ctDNA-negative patients. Thus, these preliminary findings highlight the reliability of ctDNA as valuable platform for early detection of recurrent disease as surrogate tool to improve survival outcomes. Therefore, ctDNA detection allowed the definition of a high-risk group of patients who may potentially benefit from immediate intervention after RC opening the door to the concept of molecular residual disease.

CONCLUSIONS

Emerging molecular biomarkers has recently gained attention as a magnificent option for disease diagnosis, follow-up, prognosis, and for treatment decision-making but need further validations in prospective trials before entering into the clinical daily practice.

Recent developments in MIBC-specific highly sensitive assays such as NGS, ddPCR, enzyme- or RNA-based platforms for molecular subtyping or profiling of CTCs, and analysis and detection of cf/tDNA, suggest that these tools may have future clinical applications in MIBC clinical scenario. Overall, despite this enormous potential, real-world application is hindered by lack of standardization, and poor reproducibility. Moreover, these data seem even more immature in the TMT setting.

Molecular subtyping seems attractive as it represents the mirror of biological and clinical inter-patient heterogenicity in response to radical treatments and systemic agents potentially allowing a comprehensive pretreatment counselling. However, it must be considered the dynamic ecosystem of tumor microenvironment. Here, changes in biomarker landscape from matched pretherapy and post-therapy tumor samples reveal the complex interplay between the disease, immune system, and microenvironment and how much tumor cells can adapt and respond to therapeutic stress.

Liquid biopsies might potentially guide such treatment decisions in MIBC patients, but to date studies are still immature demonstrating only a proof of principle. Particularly, cf/tDNA represents one of the most informative available tools. Currently, the most promising results have been obtained using patient-specific PCR- or NGS-based assays built on somatic variants defined in primary tumor tissue. However, to incorporate this strategy on a broad scale is challenging due to the underlying problem of the lack of hotspot mutations in MIBC hampering the development of a one-size-fits-all strategy based on somatic mutations in cf/tDNA. A deeper understanding of molecular MIBC biology is a crucial step to find a predictive signature that can be used to select appropriate treatments towards the convergence of precision oncology with both local treatments and conventional or novel systemic agents.

AUTHOR, PUBLICATION,YEAR	STUDY DESIGN	COHORT	FOLLOW - UP MEDIAN/ MEAN (RANGE)/SD	TREATMENT
Van Allen, 2015(19)	Prospective	50	351 días (SD, 363,2)	Neoadjuvant CG Neoadjuvant ddMVAC Neoadjuvant ddCG Neoadjuvant CG + Sunitinib
Iyer, 2018(20)	Prospective	49	24 months	Neoadjuvant ddCG
Desai, 2016(21)	Retrospective	46	26 meses (5 - 115)	TMT
Plimack, 2015(22)	Prospective	95	16,7 months 28,3 months	ddCG MVAC
Necchi, 2020(25)	Prospective	114	13,2 months	Neoadjuvant Pembrolizumab
Bandini, 2020(26)	Prospective	112	NA	Neoadjuvant Pembrolizumab
Powles, 2019	Prospective	95	13,1 months	Neoadjuvant Atezolizumab
van Dijk, 2020(28)	Prospective	24	8,3 months	INeoadjuvant Ipilimumab + Nivolumab
Galsky, 2021(29)	Prospective	76	13,7 months (2,5 – 24,0)	Nivolumab + CG y Nivolumab (4 months)
Magliocco, 2017(30)	Prospective	465	NA	TMT
Walker, 2019(31)	Prospective	353	NA	TMT
Groenendijk, 2016(32)	Prospective	94	NA	Neoadyuvante GC Neoadyuvante MVAC
Yang, 2018(33)	Retrospective	52	NA	neoadyuvante CG
Yuen Teo, 2020(35)	Retrospective	72	38,4 meses	CG neoadyuvante
van Rhijn, 2020(34)	Retrospective	1000	4,5 years (2,2 – 7,5)	RC and Cisplatin-based AC
Choi, 2014(37)	Retrospective	18	37,2 meses	Neoadjuvant CG
Seiler, 2017(38)	Retrospective	269	35 months (16 - 54)	CG MVAC RC alone
Necchi, 2020(42)	Retrospective Prospective	140 84	8 months (5 – 13,5) 18,4 months (12 – 22,4)	Neoadjuvant CG Neoadjuvant Pembrolizumab
Gao, 2020(43)	Prospective	28	19,2 months	Neoadjuvant Durvalumab + Tremelimumab
Grivas, 2020(39)	Retrospective	234	12 months	Neoadjuvant CG
Efstathiou, 2019(44)	Retrospective	136 223	3,5 years (2,1 – 5,0)	TMT Neoadjuvant CG
Soave, 2017(45)	Prospective	185	31 months	Upfront RC receiving adjuvant CG or MVAC
Rink, 2012(46)	Prospective	100	16 months (1 - 45)	Upfront RC
Christensen(49), 2019	Prospective	68	21 months	Neoadjuvant Atezolizumab
Powles, 2019(27)	Prospective	40	13,1 months (9,5 – 13,5)	Neoadjuvant Atezolizumab
Bellmunt, 2021(50)	Prospective	581	23,0 meses	Atezolizumab adyuvante vs observación tras CR

Abbreviations are as follows: SD: standard deviation; CG: Cisplatin-Gemcitabine; ddMVAC: dose-dense Methotrexate, Vinblastine sulfate, Doxorubicin hydrochloride (Adriamycin), and Cisplatin; ddCG: dose-dense Cisplatin-Gemcitabine; ERCC2: Excision Repair Cross Complementing 2; pCR: pathological complete response; pDS: pathological downstaging; NAC: neoadjuvant chemotherapy; TMB: Tumor Mutational Burden; NA: not available; RFS: recurrence-free survival; TMT: trimodal therapy; ATM: Ataxia Telangiectasia Mutated-1; RB1: RB transcriptional corepressor-1; FANCC:

Table 1. Molecular biomarkers of response to local treatments and systemic agents in patients with MIBC

Next →

BIOMARKER	SOURCE	CLINICAL ENDPOINT
ERCC2	Tissue	ERCC2 mutations were associated with pCR and/or pDS to Cisplatin-based nac.
ERCC2	Tissue	ERCC2 status was associated with pDS (< pT2N0) and better 2-year RFS.
ERCC2	Tissue	ERCC2 mutation status was associated with RFS.
ATM, RB1, FANCC	Tissue	ATM, RB1, FANCC alterations predicted pathologic response, better OS and PFS.
PD-L1, CPS, TMB	Tissue	TMB and CPS were associated with both pCR (pT0N0) and pDS (≤pT1)
TMB	Tissue	TMB was not independently associated with pCR (pT0N0)
TMB, T-CD8+ infiltration	Tissue	TMB was not associated with pCR (pT0N0). High CD8+ T-cell infiltration was associated with pCR (pT0N0)
TMB, PD-L1	Tissue	High TMB and higher rate of PD-L1 positivity among patients who achieved pCR (pT0N0 or pTis/pTaN0) among cT2-4N0M0 (58%) and cT2-4aN1-3M0 (42%) MIBCs.
TMB, ERCC2	Tissue	High TMB and mutant ERCC2 were associated with cCR (normal cytology, imaging, and cT0/Ta) or pT0 (among patients receiving RC).
MRE11	Tissue	Low nuclear/cytoplasmic MRE11 expression ratio correlated with worse CSS.
MRE11	Tissue	Low MRE11 staining failed to be correlated with worse CSS.
ERBB2	Tissue	ERBB2 mutation status was correlated with pCR (pT0N0).
ERBB2 FGFR3 PIK3Ca	Tissue	ERBB2, FGFR3, PIK3Ca alterados en pacientes con RPc(pT0N0).
Molecular subtypes	Tissue	ERBB2, FGFR3, PIK3Ca were more commonly altered in patients who achieved pCR (pT0N0).
FGFR3	Tissue	FGFR3 mutations were associated with favorable pathological features and good DSS.
Molecular subtypes	Tissue	pDS (<pT1) response rate was 0% in p53-like, 40% basal-like, 67% luminal-like.
Molecular subtypes	Tissue	Claudin-low and Luminal-infiltrated were associated with OS and pDS.Basal-subtype correlated with worse OS.
Molecular Subtypes	Tissue	Molecular subtypes were not associated with Cisplatin-based NAC. The Immne190 signature was associated with pCR and PFS.
Molecular subtypes	Tissue	Four-genes immune-enriched signature was correlated with pDS and pCR in Cisplatin-ineligible patients harboring high-risk MIBC features.
Molecular subtypes	Tissue	NE-like subtype exhibited worse CSS.
Molecular subtypes	Tissue	Higher immune infiltration in MIBC is associated with improved DSS after TMT, whereas higher stromal infiltration is associated with shorter DSS after NAC and RC.
CTCs	Blood	CTCs presence was an independent predictor for DSS, CSS, OS.
CTCs	Blood	CTCs were a powerful predictor of OS, RFS, CSS..
ctDNA	Plasma	ctDNA before NAC was predictor of worse RFS and OS. No pCR was observed in ctDNA-positive patients and all pT0 patients were ctDNA-negative.
ctDNA	Plasma	Neoadjuvant Atezolizumab was associated with a reduction in ctDNA levels in patients who achieved a response (pDS or pCR).
ctDNA	Plasma	Pacientes ctDNA-positivo mejor SLE con atezolizumab adyuvante frente a observación. Los pacientes ctDNA-positivo tras CR peor SG, pero mejor SG si tratados con atezolizumab que con observación

FA Complementation Group C; OS: overall survival; PFS: progression-free survival; PD-L1: Programmed cell Death-Ligand 1; CPS: combined positive score assessment;; MRE11: meiotic recombination 11 homolog; CSS: cancer-specific survival; ERBB2: Erb-B2 Receptor Tyrosine Kinase 2; FGFR3: Fibroblast Growth Factor Receptor 3; PIK3Ca: Phosphatidylinositol 4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; DSS: disease-specific survival; RC: radical cystectomy; AC: adjuvant chemotherapy; CTCs: circulating tumor cells; cfDNA: circulating cell-free DNA; ctDNA: circulating tumor DNA

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