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Advanced/metastatic bladder cancer: current status and future directions

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Abstract. – In 2015 bladder cancer was the fourth most frequent malignancy and the eighth cause of death for cancer. At diagnosis, about 30% of bladder cancer (BC) patients present a muscle-invasive bladder cancer (MIBC) and 5% a metastatic bladder carcinoma (MBC). For fit MBC patients, combination chemotherapy (CC) is the standard of care for first-line treatment. CC includes both the treatment with methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) either the classical or the dose-dense MVAC regimen, and the doublet therapy with cisplatin and gemcitabine (CG). Median progression free survival (PFS) was 7 months and median overall survival (OS) was 15 months.

The present review provides an update on the management of MBC, with focus on target therapies, immune checkpoint inhibition, looking for prognostic and predictive factors.

Key Words:
Bladder cancer, Chemotherapy, Prognostic factors, Immunotherapy.

Introduction

In 2015 bladder cancer was the fourth most frequent malignancy and the eighth cause of death for cancer. At diagnosis, about 30% of bladder cancer (BC) patients present a muscle-invasive bladder cancer (MIBC) and 5% a metastatic bladder carcinoma (MBC)[1-3]. For fit MBC patients, combination chemotherapy (CC) is the standard of care for first-line treatment. CC includes both the treatment with methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) either the classical or the dose-dense MVAC regimen, and the doublet therapy with cisplatin and gemcitabine (CG). Median progression free survival (PFS) was 7 months and median overall survival (OS) was 15 months[4,5]. MVAC treatment registered a survival advantage, but an increased toxicity limited their clinical use[6-8]. Although BC is considered a chemo sensitive tumor, patients with advanced or metastatic disease (MBC) have an estimated risk of relapse after cisplatin based treatment of 30-40% and 100% respectively[4,9-12]. For unfit patients, not eligible for a first-line cisplatin-containing chemotherapy because of their poor performance status (PS) and/or comorbidities there is no clear standard treatment but a carboplatin-based regimen or a single agent therapy are considered acceptable alternatives, according to national and international guidelines[13-15]. To date, for patients who recur or are refractory to first-line therapy there is no standard therapy. After platinum-based chemotherapy failure, the only chemotherapeutic agent approved in Europe is vinflunine (VFL). In the eligible population, VFL treatment provides a 2-month prolongation in median OS, without any detrimental effect on quality of life[16-18]. Re-challenging cisplatin-sensitive patients if progression occurs at least 6-12

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months after first-line cisplatin-based combination, or taxanes, or gemcitabine alone or in combination showed modest clinical efficacy with an ORR of 5% to 20% and a median PFS of only 3 to 4 months[19]. The prognosis of these patients remains poor and there no new drugs approved in recent years. A plethora of published studies have described different molecular pathways involved in bladder carcinogenesis. Deletion, mutation, or aberrant methylation of tumor suppressor genes such as TP53, RB1, CDKN2A, and PTEN and activation, mutation, or overexpression of oncogenes such as FGFR3, Her2, and CCND1 are commonly associated with BC tumorigenesis, progression and treatment resistance[20-23]. Expression of a number of proangiogenic factors, including HIF-1, VEGF, bFGF, IL-8 and MMPs, as well as anti-angiogenic factor TSP-1, was associated with BC progression and aggressiveness. Immunotherapy has opened new insights, particularly with the discovery of immune checkpoint inhibition (anti CTLA-4, anti PD1-PDL1/2)[24]. The present review provides an update on the management of MBC, with focus on target therapies, immune checkpoint inhibition, looking for prognostic and predictive factors.

Targets and target Therapies in Bladder Cancer

Angiogenesis Inhibitor Drugs

Vascular endothelial growth factor (VEGF) family is often overexpressed in bladder cancer and correlated with tumor progression, recurrence rate and poor prognosis. However, the results with antiangiogenesis agents have not been encouraging[25-28].

Bevacizumab

Bevacizumab (Bev) is a humanized monoclonal antibody that binds vascular endothelial growth factor (VEGF), the key driver of vasculogenesis and angiogenesis, and thereby inhibits the binding of VEGF to its receptors, Flt-1 (VEGFR-1) and KDR (VEGFR-2), on the surface of endothelial cells. Two single arm phase II study evaluated Bev in combination with GC in chemotherapy-naive patients with MBC. The RR was 72% and 49%, the mPFS was 8.2 and 6.5 months, with a mOS of 19.1 and 13.9 months respectively[29,30]. Considering the good disease control compared to historical controls, there is an ongoing phase III trial of GC with or without bevacizumab as first-line treatment and it will define the role of bevacizumab in this setting[31].

Aflibercept

Aflibercept is a recombinant fusion protein that binds VEGF-A, PlGF and VEGF-B. It was well tolerated but had limited activity in patients with platinum-pretreated MBC, with a RR of 4.5% and an mPFS of 2.79 months. No other trials are ongoing[32].

Sunitinib

Sunitinib, a multiple tyrosine kinase receptors inhibitor, including VEGFR, c-MET and PDGFR α was investigated as single agent in first-line therapy for patients with MBC, unfit for cisplatin-based chemotherapy. The RR was 8% with an mPFS of 4.8 months and a mOS of 8.1 months[33]. In a phase II trial, 77 cisplatin-refractory patients received sunitinib, 45 at standard schedule (50 mg daily for 4 weeks on and 2 weeks off) and 32 at alternative schedule (37.5 mg daily continuously). The RR was 7% and 3% respectively. The mPFS (2.4 and 2.3 months, respectively) and the mOS (7.1 and 6.0 months, respectively) were similar in both cohorts[34]. Sunitinib was combined with CG in 36 chemotherapy naive patients with MBC. The RR was 49%. Grade 3 to 4 hematologic toxicities occurred in 70% of patients and it has resulted in the early closure of the study[35]. In a randomized phase II trial sunitinib was given as maintenance therapy to 54 patients with MBC who had achieved stable disease (SD) or a partial response (PR) or complete response (CR) after 4 to 6 cycles of chemotherapy. They were randomized to receive sunitinib at a dose of 50 mg per day (28 days on and 14 days off) or placebo. Sunitinib did not improve 6-months PFS compared with placebo. Indeed the 6-months progression rate was 72% *vs.* 64% and the mPFS was 2.9 months *vs.* 2.7 months for the sunitinib *vs.* placebo arms, respectively[36].

Sorafenib

Sorafenib, a VEGFR 1-3, PDGFR-II, RAF-1 and B-Raf inhibitor showed disappointing results in MBC. A phase II trial evaluated sorafenib at standard schedule in pre-treated MBC. The mPFS was 2.2 months and mOS was 6.8 months, without objective responses. Common grade 3 toxicities included fatigue and hand-foot syndrome[37]. The same results were achieved in 17 chemo-naïve patients with MBC treated with sorafenib 400 mg twice daily.

There were no objective responses. The TTP was 1.9 months and mOS was 5.9 months[38]. In a randomized phase II trial first-line chemotherapy with gemcitabine (1250 mg/m^2 on days 1 and 8) and cisplatin (70 mg//m^2 on day 1) repeated every 21 days, was administered to 40 patients in association with sorafenib (400 mg twice daily) and to 49 patients in association with placebo (two tablets twice daily) on days 3-21 until disease progression or unacceptable toxicity. In the sorafenib arm, there was a RR of 52.5%, a mPFS of 6.3 months and a mOS of 11.3 months, not significantly different from the placebo arm. The addition of sorafenib to standard chemotherapy showed acceptable toxicity, but the study was closed prematurely because of slow recruitment and failed to show a 4.5 months improvement in PFS[39]. Sorafenib in combination with low-dose gemcitabine and paclitaxel (LD-GPS) was evaluated in cisplatin-resistant MBC. CR, PR and SD were found in 0 (0.0 %), 1 (5.0%), and 13 patients (65%), respectively. The median (interquartile range) period of OS after starting of this therapy was 7 (5-11) months. Three patients (15.0%) stopped therapy because of grade 3 fatigue and hand-foot reactions. LD-GPS therapy was well tolerated[40]. We are waiting the results of a phase II trial of Gemcitabine, Carboplatin, and Sorafenib in Chemotherapy-naive patients with MBC x 6 cycles then maintenance sorafenib alone[41]. An ongoing Phase I Study evaluated Sorafenib in addition to Vinflunine in pretreated MBC[42].

Pazopanib

Pazopanib, a multi-target tyrosine kinase inhibitor of VEGFR-1, -2, and -3, PDGFR-α and -β, and c-KIT, did not show significant activity in patients with MBC. 19 patients refractory to one prior systemic therapy, were enrolled in a phase II study and received pazopanib at a dose of 800 mg orally for a 4-week cycle. Most common toxicities were anemia, thrombocytopenia, leukopenia, and fatigue. There were no objective responses, with a mPFS of 1.9 months[43]. A similar phase II study, evaluated pazopanib at standard schedule in 41 cisplatin-refractory patients. The RR was 17%, with a mPFS of 3 months and mOS of 5 months. The most frequent treatment-related grade 3 adverse events were hypertension, fatigue and gastrointestinal and vaginal fistulisations[44]. There are several ongoing trials with pazopanib, for example: pazopanib in combination with vinflunine in patients with MBC after failure of platinum-based treatment[45], gemcitabine and pazopanib in chemotherapy naïve patients with MBC, ineligible for Cisplatin-based Chemotherapy[46] and paclitaxel plus pazopanib in patients unfit for CDDP[47].

Vandetanib

Vandetanib, an oral inhibitor of VEGFR, EGFR and RET, showed a limited activity in MBC without significant improvement in RR, PFS and OS. A randomized phase II trial evaluated the efficacy of docetaxel in combination or not with vandetanib at dose of 100 mg in 142 cisplatin-refractory patients with MBC. The mPFS was 2.56 months for the docetaxel plus vandetanib arm *vs.* 1.58 months for the docetaxel plus placebo arm. ORR and OS were not different between both arms. Grade 3 or higher toxicities were more commonly seen in the docetaxel plus vandetanib arm and included rash/photosensitivity (11%) and diarrhea (7%). Among 37 patients who crossed over to single-agent vandetanib, ORR was 3% and OS was 5.2 months[48].

Ramucirumab

Ramucirumab is a fully humanized monoclonal antibody. It acts blocking the binding of VEGF to VEGFR-2. It was evaluated in a randomized three-arm phase II trial compared docetaxel 75 mg/m^2 on day 1 of a 3-week cycle (Arm A), docetaxel 75 mg/m^2 plus ramucirumab 10 mg/kg on day 1 of a 3-week cycle (Arm B) and docetaxel 75 mg/m^2 on day 1 plus icrucumab 12 mg/kg on days 1 and 8 of a 3-week cycle (Arm C) until PD or unacceptable toxicity. A total of 140 MBC patients were enrolled. The mPFS was significantly longer in arm B than arm A (5.4 months; 95% CI, 3.1 to 6.9 months *vs.* 2.8 months; 95% CI, 1.9 to 3.6 months). Arm C did not experience improved PFS *vs.* arm A (1.6 months; 95% CI, 1.4 to 2.9; stratified hazard ratio, 0.863; 95% CI, 0.550 to 1.357; $p = 0.5053$). The most common grade 3 or worse adverse events (arms A, B, and C) were neutropenia (36%, 33%, and 39%), fatigue (13%, 30%, and 20%), febrile neutropenia (13%, 17%, and 6.1%), and anemia (6.7%, 13%, and 14%, respectively). The addition of ramucirumab to docetaxel prolonged PFS in second-line treatment. The addition of icrucumab to docetaxel did not experience improved PFS. OS was not significantly different between the two arms[49]. An ongoing phase III trial (RANGE) is comparing docetaxel alone vs ramucirumab plus docetaxel[50].

PI3K/AKT/mTOR

Mutations, copy number alterations, or RNA expression changes affecting the PI3K/AKT/mTOR pathway were observed in 42% of MBC, including activating point mutations in PIK3CA (17%), mutation or deletion of TSC1 or TSC2 (9%), and overexpression of AKT3 (10%) and it represents a rational target for therapeutic intervention[51,52].

Everolimus

Everolimus is a selective mTOR inhibitor. In a single-arm, non-randomized phase II study 45 patients with MBC progressing after one to four cytotoxic chemotherapy regimens received everolimus 10 mg orally once daily continuously. There were 23 of 45 (51%) patients who were progression-free at 2 months with a mPFS of 2.6 (95% CI, 1.8-3.5) months and a median OS of 8.3 (95% CI, 5.5-12.1) months[53]. We are waiting the results of a single arm, multi-center phase II trial evaluate paclitaxel plus everolimus in MBC after failure of prior platin-based chemotherapy[54] and a phase II trial evaluated everolimus alone or everolimus plus paclitaxel as first-line therapy in cisplatin-ineligible patients with MBC[55].

Temsirolimus

Temsirolimus, a selective inhibitor of mTOR, seems to have poor activity in patients with MBC after failure of platinum containing first-line therapy. In a phase II, trial 15 patients with MBC progressed after platinum containing regimens received weekly 25 mg of temsirolimus for 8 weeks as second-line therapy. Temsirolimus was well tolerated. mTTP was 2.5 months, mOS was 3.5 months. No sufficient benefit on OS was observed and the study was early stopped[56]. An ongoing phase I/II trial evaluatedthe side effects and best dose of sirolimus when given together with cisplatin and gemcitabine hydrochloride[57].

Agents Targeting Epidermal Growth Factor Receptor (EGFR)

The ErbB family of tyrosine kinase receptors are overexpressed in various epithelial tumors, including breast, lung, gastrointestinal, cervical, and bladder cancer. It is involving in tumorogenesis, disease progression and resistence to treatment[21].

EGFR (HER1)

EGFR signaling pathway correlates with advanced tumor stage, higher tumor recurrence rate, progression and poor overall survival[58]. Higher levels of EGFR appear to correlate with the basal-like histologic subgroup of bladder cancer and correlates with cisplatin resistance[59].

Cetuximab

Cetuximab, a chimeric monoclonal IgG1 antibody that is specifically directed against the EGFR, although it had limited activity as single agent in pretreated MBC, it appears to increase the activity of paclitaxel. In a randomized phase II study 39 patients with MBC progressed after one line of chemotherapy were randomly assigned to 4-week cycles of cetuximab 250 mg/m^2 with or without paclitaxel 80 mg/m^2 per week. The RR was 25%, the mPFS was 16.4 weeks and the mOS was 42 weeks in the combination arm[60]. A randomized phase 2 trial evaluated the role of gemcitabine/cisplatin with or without cetuximab in patients with MBC. The mPFS was 8.5 months for arm A (95% CI = 5.7-10.4 months) and 7.6 months for arm B (95% CI = 6.1-8.7 months). The median OS was 17.4 months for arm A (95% CI = 12.8 months to unreached) and 14.3 months for arm B (95% CI = 11.6-22.2 months). The most common grade 3/grade 4 adverse events in both arms were myelosuppression and nausea. An increased soluble E-cadherin level after cycle 2 correlated with a higher risk of death. GC plus cetuximab was feasible but was associated with more adverse events and no improvements in outcomes[61].

Panitumumab

Panitumumab is a recombinant, fully human IgG2 monoclonal antibody that binds with high affinity and specificity to the human EGFR. A randomized phase II study by the German Association of Urological Oncology compared CG and panitumumab *vs.* CG alone as first-line therapy for patients with MBC but this study was closed due to insufficient recruitment[62].

Gefitinib

Gefintinib, an orally active selective EGFR TKI, was evaluated in a phase II study as single agent in 31 MBC patients progressed after platinum-based chemotherapy. The RR was 3%, mPFS was 2 months and mOS was 3 months[63]. The combination of gefitinib to the first-line therapy with CG did not appear to improve RR

or OS *vs.* CG alone, with a RR of 42.6%, a mTTP of 7.4 months and a mOS of 15.1 months[64]. A phase II trial of weekly docetaxel (arm A) *vs.* weekly docetaxel in combination with gefitinib followed by gefitinib alone as consolidation therapy for MBC (arm B), was recently terminated. The study did not meet its primary endpoint; no patients were free from progression at 9 months from the start of consolidation therapy. mPFS in arm A was 3.7 *vs.* 4.4 months in arm B with a mOS of 18.0 (arm A) *vs.* 16.6 months (arm B)[65].

HER2

Polysomy 17 and HER-2/neu gene amplification or protein overexpression have been associated with advanced disease, poor prognosis and resistence to chemiotherapy and radioterapy. In BC, HER-2/neu expression has been reported over 10 years and range from 2% to 71%, according to the use of various techniques and criteria[66,67].

Trastuzumab

Trastuzumab is a monoclonal antibody that interferes with the HER2/neu receptor. In a phase II trial 44 untreated patients with HER2-positive MBC received trastuzumab 4 mg/kg loading dose followed by 2 mg/kg on days 1, 8, and 15 in combination with paclitaxel 200 mg/m^2 on day 1, carboplatin AUC 5 on day 1 and gemcitabine 800 mg/m^2 on days 1 and 8. The RR was 70%, mTTP was 9.3 months and mOS was 14.1 months. Cardiac toxicity rates were higher than projected, but the majority were grade ≤ 2, so this treatment can be considered feasible[68]. Another phase II trial evaluated the effectiveness and feasibility of the association of trastuzumab with chemotherapy (CG or Carboplatin and gemcitabine) in HER2-expressing MBC. No difference was observed in overall RR, PFS, and OS between the chemotherapy-alone arm and the chemotherapy-plus-trastuzumab arm[69]. An Open-label Pilot study investigating standard CG chemotherapy in combination with trastuzumab in the first-line setting closed enrollment early[70]. Another study with single-agent trastuzumab in the second-line treatment closed early due to poor recruitment[71].

Lapatinib

Lapatinib is an orally dual tyrosine kinase inhibitor. Lapatinib demonstrated an improvement in OS in a subset of patients with tumors overexpressing EGFR and/or HER-2. In a single-arm phase II, study 59 patients with MBC progressed on platinum-based chemotherapy received lapatinib until PD or unacceptable toxicity. This treatment led to an RR of 1.7%, a mTTP of 2 months and a mOS of 4.1 months. In a subgroup analysis, clinical benefit was correlated with EGFR overexpression and HER-2 overexpression. Lapatinib was well tolerated[72]. A phase II/III trial compared maintenance lapatinib (L) *vs.* placebo (P) in 232 patients with HER1/HER2 positive MBC who achieved clinical benefit after completing first-line chemotherapy. The PFS for L and P was 4.6 months (95% CI: 2.8-5.4) and 5.3 months (95% CI: 3.0-5.9), respectively [HR: 1.04 (95% CI: 0.79-1.39) $p = 0.77$]. The OS for L and P was 12.6 months (95% CI: 9.5-16.2) and 11.9 months (95% CI: 10.6-15.8), respectively [HR = 0.98 (95% CI: 0.71-1.35) $p = 0.89$). Maintenance lapatinib does not improve outcomes in HER1 or HER2 positive MBC patients[73]. In a phase I trial 18 patients received lapatinib at dose of 750-1,250 mg in combination with CG. This treatment appeared safe and tolerable[74]. Pending are the results of the phase II study of docetaxel and lapatinib in MBC as second line treatment[75].

Afatinib

Afatinib is an oral, irreversible inhibitor of the EGFR and HER2. Afatinib showed significant activity in patients with platinum-refractory MBC with HER2 or ERBB3 alterations. In a phase II trial, patients with platinum-refractory MBC received afatinib 40 mg/day continuously until PD or intolerance. No unexpected toxicities were observed. The median time to progression/discontinuation was 6.6 months for patients with HER2/ERBB3 alterations *vs.* 1.4 months in patients without these alterations. The potential contribution of ERBB3 to afatinib sensitivity is novel[76]. Due to these results, afatinib deserves further investigation in molecularly selected MBC.

FGFR

The FGF/FGFR signaling axis comprises of about 20 ligands that bind to four highly conserved trans-membrane tyrosine-kinase receptors (FGFR1, 2, 3 and 4). Amplifications of the FGFR1 gene have been found in 9-10%, FGFR2 gene in 0.8% and FGFR3 gene in 3-5% of BC. FGFR3 has been shown to harbour activating mutations in 38-66% of non-invasive BC and in 15-20% of invasive BC. The prognostic value of FGFR3 activating mutations and overexpression of wild-type form are not yet known, even if it seems to correlate with good prognosis, low stage and low-grade[77].

Dovitinib

Dovitinib is an inhibitor of FGFR1, FGFR2, and FGFR3. A phase II trial evaluated dovitinib at dose of 500 mg/day on a 5-days-on/2-days-off schedule in patients with MBC platinum-refractory. Forty-four MBC patients, progressed after one to three platinum-based and/or combination chemotherapy regimens were classified as having mutant (FGFR3 (MUT); n=12), wild type (FGFR3 (WT); n=31), or unknown (n=1) FGFR3 status. Although there were difficulties in evaluating mutation status, dovitinib had limited single-agent activity in patients with MBC regardless of FGFR3 mutation status. mPFS was 3 months in the FGFR3 mutational group and 1.8 months in the FGFR3 non-mutational group. The most common grade 3/4 adverse events, included thrombocytopenia (9%), fatigue (9%), and asthenia (9%)[78]. Further studies are needed to understand the role of FGFR3 inhibition in advanced BC treatment.

BGJ398

BGJ398 is a FGFR inhibitor. Patients with activating FGFR3 mutations/fusions and prior platinum-based chemotherapy received BGJ398 at the dose of 125 mg once daily, 3 weeks on/1 week off. The study evaluated ORR and safety also. BGJ398 monotherapy was well tolerated and had encouraging activity in heavily pretreated patients with MBC. The 36% ORR observed in these patients is notable given their poor prognosis and limited therapeutic options[79].

MET/HGF1 Pathway

Cabozantinib

Cabozantinib is a potent VEGF-2 and MET (HGF1) dual TKI. MET signaling acts maintaining the VEGF signaling in tumour angiogenesis, invasion and proliferation. An ongoing phase II study is evaluating cabozantinib 60 mg daily in 3 cohorts patients: 1) relapsed or refractory MBC, 2) bone-only relapsed or refractory MBC, and 3) metastatic rare bladder histology. Preliminary results showed: a mPFS of 3.7 months (95% CI: 2.3-6.5) and a mOS of 8.2 mos (95% CI: 5.2-10.3) in the cohort 1 (41 patients) respectively; a mPFS of 5.3 mos. (95% CI: 1.8-8.3) and a mOS of 9.3 mos. (95% CI: 3.6-12.5) in the cohort 2 (4 patients) respectively; a mPFS of 2.9 mos. (95% CI: 1.8-3.7) and a mOS of 4.6 mos. (95% CI: 2.6-8.0) in the cohort 3 (10 patients) respectively[80]. Peripheral blood samples were obtained from patients with advanced/refractory MBC undergoing treatment with cabozantinib under the clinical trial NCT01688999. Myeloid-derived suppressor cells (MDSC) and regulatory T cells (Tregs) were measured in 24 patients at baseline and after 2 cycles of continuous cabozantinib treatment. Patients with low Tregs at baseline had an improved PR rate, PFS and OS. Tregs decreased with cabozantinib treatment. Programmed death 1 (PD-1) expression in Tregs increased after cabozantinib. The percent MDSC did not change with treatment. MDSC CD40 expression increased after cabozantinib treatment compared to baseline. Treg levels prior to cabozantinib treatment are predictive of therapeutic responsiveness and OS. Changes in Treg PD-1 expression and MDSC CD40 expression may be prognostic markers in patients with advanced/refractory MUC treated with cabozantinib[81].

Immune Checkpoint Pathway

In the last years, immunotherapy is emerging as a new therapeutic strategy to enhance the host immunity against cancer cells. Immune therapy focusing on novel agents that target proteins in the immune checkpoint regulation pathway (Programmed cell death protein 1 [PD-1], PD ligand 1 [PD-L1], Cytotoxic T lymphocyte-associated protein 4 [CTLA-4]). This kind of treatment has been very successful in a variety of solid tumors, including metastatic melanoma, lung cancer (NSCLC), renal cancer (RCC)[82]. Bladder cancer is emerging as immunogenic tumour and several trials have been concluded or are ongoing with promising results[83].

Ipilimumab

Ipilimumab is a fully human monoclonal antibody that blocks the interaction between B7 (B7-1 and B7-2 are homologous costimulatory ligands expressed on the surface of antigen presenting cells) and CTLA-4 (cytotoxic T-lymphocyte antigen 4), causing a negative inhibition that increases the activation and proliferation of T-lymphocytes against cancer cell. A phase II trial of CG plus ipilimumab as first-line treatment for patients with MBC is ongoing[84]. A preliminary report[85] showed that a phased schedule of CG plus ipilimumab (2 cycles CG alone followed by 4 cycles of CG + ipilimumab, followed by ipilimumab maintenance administered every 3 months) was feasible in patients with MBC; mPFS was 8 months (95% CI 6.2-9.8 months) and mOS was

14.6 months (95% CI 10.5-18.6 months). The trial did not reach the primary endpoint (percentage of patients alive at 1 year). Ipilimumab induced immunomodulatory effects despite concomitant chemotherapy. Ongoing analyses are exploring the impact of GC alone, and GC plus ipilimumab, on antigen-specific T cell immunity and overall survival[86].

Atezolizumab

Atezolizumab is a fully humanized monoclonal antibody against the PD-L1, blocking the interaction with PD-1. Atezolizumab demonstrated good tolerability and a favorable safety profile compared to historical chemotherapy and received breakthrough designation status by the FDA in 2014. A multicenter, single-arm phase II trial (IMvigor210) evaluated atezolizumab (1,200 mg every three weeks) in 310 patients with locally advanced or MBC that had progressed after platinum-based chemotherapy. PD-L1 expression on tumour-infiltrating immune cells (ICs) was assessed prospectively by immunohistochemistry. The PD-L1 expression status was defined by the percentage of PD-L1-positive immune cells: IC0 (<1%), IC1 (≥1% but < 5%), and IC2/3 (≥5%). Based on independent evaluation, the objective response rates were 26% (95% CI, 18-36%) in the IC2/3 group, 18% (95% CI, 13-24%) in the IC1/2/3 group and 15% (95% CI, 11-19%) in all patients. At a median follow-up of 12 months, ongoing responses were observed in 84% of responding patients. The mOS was 11.4 months (95% CI, 9.0-not estimable) in the IC2/3 group, 8.8 months (95% CI, 7.1-10.6) in the IC1/2/3, and 7.9 months (95% CI, 6.6-9.3) in all patients. The 12-months landmark OS rate was 48% in the IC2/3 (95% CI, 38-58%) group, 39% in the IC1/2/3 (95% CI, 32-46%) group and 36% (95% CI, 30-41%) in the intent to treat population. The most common side effects were fatigue, decreased appetite, nausea, urinary tract infection, fever, and constipation. Grade 3-4 immune-mediated adverse events was noted in 5% of patients. The PD-L1 expression, the TCGA molecular subtypes, and mutation load were independently associated with response to atezolizumab[86,87]. With a longer follow up (median 14.4 mo [range 0.2-17.1]), the median OS was 11.9 months (95% CI, 9.0–not estimable) in the IC2/3 group, 9.0 months (95% CI, 7.1-10.9) in the IC1/2/3, and 7.9 months (95% CI, 6.6-9.3) in all patients. The 12-months landmark OS rate was 50% in the IC2/3 (95% CI, 38-58%) group, 40% in the IC1/2/3 (95% CI, 32-46%) group and 37% (95% CI, 30-41%) in all patients[88]. Based on these results, atezolizumab was approved by the FDA in May 2016. An ongoing Phase III (IMvigor130), multicenter, randomized, placebo-controlled, double-blind study is evaluating the safety and efficacy of atezolizumab in combination with gemcitabine/carboplatin *vs.* placebo plus gemcitabine/carboplatin in untreated patients with locally advanced or MBC[89]. A phase III (IMvigor21), open-label, multicenter, randomized study is investigating the efficacy and safety of atezolizumab compared with chemotherapy in patients with locally advanced or MUC after failure with platinum-containing chemotherapy[90]. Recently, a phase II study evaluating the safety and efficacy of first-line atezolizumab monotherapy (1200 mg fixed dose) in 123 cisplatin-ineligible patients with advanced or MBC, showed an objective response rate of 23% (95% CI 16 to 31), with a mOS of 15.9 months (10.4 to not estimable). Tumor mutation load was associated with response. These results indicate that atezolizumab is active in patients with advanced or MBC, untreated cisplatin-ineligible urothelial carcinoma[91].

Pembrolizumab

Pembrolizumab is highly selective humanized monoclonal antibody that blocks PD-1/PD-L1. The phase Ib multi-cohort KEYNOTE-012 study is evaluating pembrolizumab in patients with advanced solid tumors. An updated efficacy and safety analysis was conducted in 33 patients with recurrent or metastatic PD-L1–positive MBC. These patients, 75% of whom had prior platinum-based therapy, received pembrolizumab at dose of 10 mg/kg every 2 weeks until complete response, progression, or unacceptable toxicity. In the 28 patients with measurable disease at baseline, ORR was 25%, with 11% of CR and 14% of PR. The 12-months PFS rate was 19%; for patients with tumors positive for PD-L1 expression (defined as >1% in tumor nests or a PD-L1-positive band in stroma by a prototype immunohistochemistry assay), the ORR was 38%, with an mPFS and OS of 2 and 12.7 months, respectively. The 1-year landmark OS was 53%. PD-L1 expression correlated with response[92]. The safety analysis of KEYNOTE-012 (N = 33) showed that fatigue was the most common adverse event (18%), followed by peripheral edema (12%), and nausea (9%); 15% had grade 3-5 adverse events and 1 patient discontinued due to grade 3 rhabdomyolyses[92]. The phase III randomized KEYNOTE-045 trial compared pembrolizumab (200 mg/m^2 q3w)

vs. paclitaxel, docetaxel or vinflunine in patients with recurrent or progressive MBC, showed an OS of 10.3 months with pembrolizumab *vs.* 7.4 months with chemotherapy, HR: 0.73 (95% CI, 0.59-0.91). The benefit was observed regardless of PD-L1 expression. The ORR was also significantly improved with pembrolizumab (21.1% *vs.* 11.4%; $p = 0.0011$). The incidence of most adverse events was lower in the pembrolizumab arm *vs.* chemotherapy[93]. The phase II KEYNOTE-052 trial is investigating the efficacy of pembrolizumab in patients with advanced or MBC untreated or ineligible for cisplatin-based therapy[94]. A planned interim analysis of the first 100 patients has reported an ORR of 24.0% unselected subjects and 36.7% in those with ≥10% combined positive score (CPS; tumor and immune cell PD-L1 expression) after median 8-months follow-up. Moreover, complete responses were observed in 6.0% of all-comers and 13.3% of those with high CPS. Adverse events were common (67%), comprising mainly of fatigue (14%); 16% experienced a grade 3/4 adverse event[94]. An interesting randomized phase II trial of maintenance pembrolizumab *vs.* placebo after first-line chemotherapy in patients with MBC who have achieved at least stable disease on first-line chemotherapy is ongoing[95]. Another ongoing phase III randomized trial is evaluating pembrolizumab with or without platinum-based combination chemotherapy *vs.* chemotherapy alone in patients with advanced or MBC (PD-L1 positive or negative tumors)[96]. An ongoing phase I trial is evaluating the tolerability and the best dose of pembrolizumab in association with docetaxel or gemcitabine in patients with previously treated BC[97].

Nivolumab

Nivolumab is a fully human PD-1 immune checkpoint inhibitor antibody, showed a survival benefit in patients with melanoma, lung cancer, and renal cell carcinoma. A phase I/II CheckMate 032 study (NCT01928394), evaluated the efficacy and safety of nivolumab (3 mg/kg q2w) monotherapy in 78 pts with MBC after ≥1 prior line of platinum-based therapy. At a median follow-up of 213 days (range, 22-499), 33.3% of patients remained on therapy; primary reason for treatment discontinuation was disease progression. Median number of doses was 8.5 (range, 1-34), 70.5% received >4 doses. The mPFS was 2.8 months (95% CI 1.5-5.5) with an ORR of 24.4 (95% CI 15.3-35.4) for those with PD-L1 expression ≥ 1% on tumor cells (TC) *vs.* 26% for those with PD-L1 expression <1%, and the OS was 9.7 months for the entire population. Grade 3 or 4 treatment-related adverse events occurred in 20.5% of patients[98]. The single-arm, open-label CheckMate 275 study (NCT02387996) of nivolumab (3 mg/kg IV q2w) in patients with MBC who have received prior therapy (N = 265) demonstrated an ORR of 19.6% for the total population, 16.1% in those with low or no PD-L1 tumor expression (<1%), and 28.4% in those with PD-L1 tumor expression ≥ 5% after a median 7-months follow-up. The mPFS was 2.0 months and the mOS was 8.7 months. An 18% of patients experienced grade 3/4 adverse events (fatigue and diarrhea; 2% each) and 1% of patients experienced a grade 5 event[99]. As part of the CheckMate 032 study, the combination of nivolumab plus ipilimumab is being investigated: Cohort A (n = 26) nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg) and Cohort B (n = 104) nivolumab (3 mg/kg) plus ipilimumab (1 mg/kg). The cohort investigating the higher dose of ipilimumab had a numerically greater RR of 39% (95% confidence interval [CI], 20.2-59.4) *vs.* 26% (95% CI, 17.9-35.5) for the lower dose. The mOS was similar in both groups: Cohort A = 10.2 months (95% CI, 4.5- NR); Cohort B = 7.3 (95% CI, 5.6-11.4 months), mPFS was less than 5 months in both cohort. Adverse events were in line with those previously seen with these drugs in other tumors[100].

Durvalumab

Durvalumab is a modified human IgG1 mAb that blocks PD-L1 binding to PD-1 and CD80. A Phase 1/2 dose escalation and dose expansion study evaluated the safety and the efficacy of durvalumab at dose of 10 mg/kg IV q2w for up to 12 months. PD-L1 expression on tumor cells (TC) and tumor-associated immune cells (IC) is defined as ≥ 25% of TC or IC staining for PD-L1. The ORR was 31% in the overall population and 46% in the PD-L1 high (defined as TC or IC ≥ 25%) subgroup *vs.* 0% in the PD-L1 low/neg subgroup (defined as TC and IC <25%). The median duration of response has not yet been reached (range: 4-49 weeks), and responses were ongoing in 12 of 13 patients at the time of publication. The most common adverse events were fatigue (13%), diarrhea (10%), and decreased appetite (8%), and grade 3 adverse events occurred in 5% of patients; there were no grade 4 or 5 events[101]. The combination of durvalumab plus the CTLA-4 inhibitor, tremelimumab *vs.* standard-of-care chemotherapy in patients with MBC, is ongoing (DANUBE)[102].

Avelumab

Avelumab is a fully human anti-PD-L1 IgG1 antibody under clinical investigation in multiple cancers. Fourty-four patients with MBC unselected for PD-L1 expression received avelumab 10 mg/kg IV biweekly until progression, unacceptable toxicity, or withdrawal. At a follow-up of 11 months ORR was 18.2% (8 pts; 95% CI: 8.2, 32.7), DCR was 56.8%. PD-L1 expression was evaluable in 35 patients. Using a ≥ 5% cut off for tumor cell staining, 12/35 [34.3%] were PD-L1+; ORR was 50.0% in PD-L1+ patients (6/12; 95% CI: 21.1, 78.9) *vs.* 4.3% in PD-L1- patients (1/23; 95% CI: 0.1, 21.9). PFS rate at 24 weeks was 58.3% (95% CI: 27.0, 80.1) in PD-L1+ patients *vs.* 16.6% (95% CI: 4.2, 36.0) in PD-L1-. ORR in patients ± baseline visceral metastasis was 18.5% (5/27) and 17.6% (3/17), respectively. OS at 12 months was 50.9% (95% CI: 32.6, 66.6) for the overall population[103]. A randomized phase 3 trial of avelumab *vs.* BSC as a maintenance treatment in locally advanced or MBC patients whose disease did not progress after completion of first-line platinum-based therapy is ongoing[104].

Discussion

In the past 30 years, treatment for MBC has not advanced beyond cisplatin-based combination chemotherapy and to date no new drugs have been approved in Europe for these patients setting. Overall survival remains poor, with mOS of 14-15 months, and there is no recognized second-line therapy, the only chemotherapeutic agent approved in Europe is vinflunine (VFL). The increasingly detailed knowledge of the molecular mechanisms underlying the development of MBC has resulted in the identification of important genes and pathways, particularly those involving the PI3-kinase/AKT/mTOR, CDKN2A/CDK4/CCND1 and RTK/RAS pathways, as well as ERBB2 (Her-2), ERBB3 and FGFR3. Their role as prognostic/predictive value is unclear and could represent valuable therapeutic targets. Unfortunately, no TT has achieved significant clinical benefits and to date no TT have been approved in this setting and none of the involved pathways can be use as prognostic or predictive markers. One of the reasons of this treatment failure is that a single biological pathway does not seem to be a dominant driver of the growth of BC. Recently a comprehensive molecular characterization of urothelial BC (TCGA analysis), identified four distinct subsets of urothelial carcinoma: (1) cluster I with a papillary morphology and FGFR3 mutations, such as overexpression of FGFR3 or FGFR3-TACC3 gene fusions; (2) clusters I and II similar to luminal A breast cancer, with high expression of GATA3 and FOXA1, E-cadherin, etc; (3) clusters I and II with ERBB2 mutation and estrogen receptor beta (ESR2) expression; (4) cluster III ('basal/squamous-like') similar to both basal-like breast cancer, and squamous cell cancers of the head and neck and lung, with overexpression of several keratin genes[21,22]. All trials investigating TT had the limit to enroll patients regardless of the cancer molecular profile without a homogeneous stratification of patients into risk groups according to validated prognostic parameters (the Bajorin criteria)[19]. A deeper knowledge of the molecular mechanisms and the opportunity to select subgroups of patients based on the molecular profile should be a starting point for planning new trials. In fact, currently all ongoing trials require as inclusion criteria the expression or not of the target in object. Previously, a report from the Bladder Cancer Advocacy Network Clinical Trials Working Group showed that the majority of contemporary clinical trials in MIBC and MBC are small, nonrandomized, phase 2 trials involving only 1 to 3 study sites. TT is explored in 58% of the trials either as single agents or in combination with cytotoxic drugs. Antiangiogenic therapies are the most common targeted therapeutic class explored, included in 48% of trials involving TT[105]. In the last 20 years, great efforts have been made to predict disease outcome and response to treatment by developing prognostic risk group using clinical-pathological factors, or testing several bio-molecular markers as predictive of treatment response. Unfortunately all of these failed to assess patient's prognosis and in predicting treatment response. Several molecular markers easily assessable by routine immunohistochemically were evaluated, but none has entered in routine clinical practice[106]. Bellmunt et al[107] conducted a first attempt of prognostic model construction in second line setting. In this study, OS differed based on: the time from previous chemotherapy (TFPC) < 3 months, Eastern Cooperative Oncology Group performance status (ECOG-PS) >0, hemoglobin (Hb) <10 g/dL and liver metastasis (LM). The median OS of four groups based on 0,1,2 and 3-4 factors was: 12.2, 6.7, 5.1 and 3.0 months, respectively[108]. The absence of prognostic patients' stratification, like the previous one, in all the trial analyzed can af-

fect and confound the interpretation of the activity of new agents (TT) in phase II trials, particularly when RR and mPFS were primary endpoints, considering the activity of TT. Recently, Pond et al[109] showed as the mPFS at 6 months (PFS6) correlates robustly with OS in second line setting, and may represent a more suitable primary endpoint than RR in phase II trials[108]. In the last years, immunotherapy is emerging as a new therapeutic strategy to enhance the host immunity against cancer cells. Immune therapy focusing on novel agents that target proteins in the immune checkpoint regulation pathway (Programmed cell death protein 1 [PD-1], PD ligand 1 [PD-L1], Cytotoxic T lymphocyte-associated protein 4 [CTLA-4]) with great survival benefit in a variety of solid tumors, including metastatic melanoma, lung cancer (NSCLC), renal cancer (RCC)[81]. Bladder cancer is emerging as immunogenic tumour and several trials are concluded or are ongoing with promising results[82]. In the 1970s, Alvaro Morales described the use of intravescical BCG for non-muscle-invasive bladder cancer (NMIBC) and demonstrated a reduction in tumor recurrence in 7 of 10 patients[110]. Therefore, BCG became a standard of care in the treatment of high-risk NMIBC after transurethral resection and it represented one of the first uses of immunotherapy in the treatment of this tumors. Malignant cells have various tumor-associated neoantigens (TAAs) complexed with MHC-I on their surface because of different genomic mutations and that make them immunogenic cells. The cancer-immunity cycle is a dynamic system in which the immune system seeks to identify and to eliminate malignant cells. Conversely cancer cells can downregulate the expression of tumor antigens (molecules that are unique to tumor cells) on cells surface so that they are no longer detected as foreign. Furthermore cancer cells can express other proteins on the their surface, that induce immune cell deactivation, or they can release cytokines, such as transforming growth factor beta (TGF-b) in the tumor microenvironment, promoting an immunosuppressive tumour microenvironment (TME), that suppress immune responses while promoting tumor cells proliferation and survival[111-115]. The involvement of immune response during bladder carcinogenesis was confirmed by Pignot et al[116] who showed as PD-1 and PD-L1 were significantly overexpressed in MIBC compared to normal bladder tissue (59.5 *vs.* 6.7% and 60.7 *vs.* 0% respectively, $p < 0.01$), whereas the proportion of overexpression was low in NMIBC (22.5% and 4.2% respectively). In contrast, a recent study found that PD-L1 expression did not differ between NMIBC and MIB and that the PD-L1 expression in tumor-infiltrating mononuclear cells was predictive of longer overall survival (OS). Overall, cancers with the high mutational burden, such as bladder cancer may contain higher number of "neo-antigens" that can be targeted by the immune system. Similarly, CTLA4 and one of its ligands CD80 were significantly overexpressed in MIBC as compared to normal bladder tissue (84.5 *vs.* 20.0% and 92.9 *vs.* 6.7% respectively, $p < 0.01$), whereas CD80 was individually overexpressed in 46.5% of NMIBC without overexpression of CTLA4[117]. Atezolizumab was the first immunotherapy approved by the FDA for the treatment of MBC in second line setting, in the era of immune checkpoint inhibitors. The phase II trial (IMvigor210) showed impressive results in second line setting with a median OS of 11.9 months (95% CI, 9.0–not estimable) in the IC 2/3 group, 9.0 months (95% CI, 7.1-10.9) in the IC 1/2/3, and 7.9 months (95% CI, 6.6-9.3) in all patients (IC 0/1/2/3) evaluated[88]. In February 2017 Pembrolizumab, another anti PD-1, was granted priority review by the FDA, as first-line treatment of patients who are ineligible for cisplatin-containing therapy and as second-line treatment for patients whose disease progressed on or after platinum containing chemotherapy. The phase III randomized KEYNOTE-045 trial compared pembrolizumab (200 mg/m^2 q3w) *vs.* paclitaxel, docetaxel or vinflunine in patients with recurrent or progressive MBC, showing an OS of 10.3 months with pembrolizumab *vs.* 7.4 months with chemotherapy, HR: 0.73 (95% CI, 0.59-0.91), representing the first agent that improves OS in the second-line setting. The same results were reached by nivolumab, and it is currently approved by FDA for the treatment of patients with MBC whose disease has progressed during a period of up to 1 year after first-line platinum-containing chemotherapy (February 2017). Other anti PD-1, like durvalumab, is under investigation. Very interesting is the antiPD-L1 avelumab that differs from the other PD-L1 inhibitors because in addition to inhibit PD-L1, it possesses antibody-dependent cell mediated cytotoxicity, which results in direct lysis of tumor cells. Currently all ongoing trials are evaluating the use of anti PD-1 or PDL-1 alone or in combination with chemotherapy. Notably, all these immunotherapeutic agents showed a favorable toxicity profile in line with those previously seen with these drugs in

other tumors, particularly no renal toxicity, considering its importance for the high incidence of renal impairment in BC.

Is the immunotherapy effective in all MBC? Is the PD-1/PD-L1 status predictive markers that will aid in the selection of patients most likely to respond to checkpoint blockade? Currently there are no clear responses and new research is needed to clarify this position. The PD-L1 expression, the TCGA molecular subtypes, and mutation load were independently associated with response to atezolizumab[86,87]. Classifying patients based on The Cancer Genome Atlas (TCGA) subtype found that immune cell PD-L1 prevalence was highly enriched in the basal subtype *vs.* the luminal subtype, while tumor PD-1 expression was seen almost exclusively in the basal subtype[21,22]. Although response to atezolizumab occurred in all TCGA subtypes, it was significantly higher in the luminal cluster II subtype than the others, suggesting that subtypes differ in other immune parameters besides PD-L1[86]. The clinical benefit of these agents, including also pembrolizumab and nivolumab, seems to be observed regardless of PD-L1 and PD-1 expression. The high mutation burden as well as frequent copy number alterations and chromosomal rearrangements, typically of BC, can explain the success of immunotherapy. The limit of the use of PD-1/PD-L1 status as a biomarker has been the heterogeneity of reported methods for assessing PD- positivity on IHC, as well as the determination of PD-1/PD-L1 status on Tumor Cells (TC) or on Tumour Associated Immune Cells (IC) or on Tumour Microenvironment[118,119]. The phase 3 trial with pembrolizumab showed the CPS PD-L1 biomarker, incorporating both tumor cell membrane and immune cell PD-L1 staining, was predictive and prognostic in platinum refractory disease[93]. In the phase 1/2 study of durvalumab was utilized an assay that measured PD-L1 expression for both TC and IC, defining PD-L1 status based on expression on TC or IC (high defined as TC or IC ≥25% and low defined as TC and IC <25%) separately. This evaluation did not result in a clear distinction between responders and non-responders. On the contrary, this analysis was predictive when looking at TC or IC expression as a combined measure[101]. Considering these data, the PD-L1 status should be conducted on both TC and IC. While the presence of PD-L1 can be predictive of response, the lack of PD-L1 expression should not preclude the use of these agents.

Conclusions

Nowadays, there are no new molecular targeted agents for treatment of MBC despite the increased understanding of the molecular mechanisms underlying BC tumorigenesis. The new immunotherapeutic agents have opened new insights, particularly with the discovery of immune checkpoint inhibition (anti CTLA-4, anti PD1-PDL1/2) that offer interesting and long-lasting results, with significant improvement in OS in heavily pre-treated MBC patients, regardless PD-L1 status. As regards the use of PD-L1 as a predictor factor, its determination on tumor sample will help in selecting responders patients, even if until the immunotherapy response mechanisms are not fully understood, it is not possible to make the right selection with a consistent risk to do not use this medication in people who may be beneficial. Several ongoing trials are evaluating immune therapy combinations and immune therapy combined with conventional chemotherapy and TT. The new goal will be the identification of clinical and molecular markers able to select patients for plan a sequential therapeutic modality.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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