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New and Promising Strategies in the Management of Bladder Cancer

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OVERVIEW

Bladder cancer is a complex and aggressive disease for which treatment strategies have had limited success. Improvements in detection, treatment, and outcomes in bladder cancer will require the integration of multiple new approaches, including genomic profiling, immunotherapeutics, and large randomized clinical trials. New and promising strategies are being tested in all disease states, including nonmuscle-invasive bladder cancer (NMIBC), muscle-invasive bladder cancer (MIBC), and metastatic urothelial carcinoma (UC). Efforts are underway to develop better noninvasive urine biomarkers for use in primary or secondary detection of NMIBC, exploiting our genomic knowledge of mutations in genes such as *RAS, FGFR3, PIK3CA,* and *TP53* and methylation pathways alone or in combination. Recent data from a large, randomized phase III trial of adjuvant cisplatin-based chemotherapy add to our knowledge of the value of perioperative chemotherapy in patients with MIBC. Finally, bladder cancer is one of a growing list of tumor types that respond to immune checkpoint inhibition, opening the potential for new therapeutic strategies for treatment of this complex and aggressive disease.

Cancer is a genetic disease.[1] A cancer cell inherits or acquires mutations that enable it to grow efficiently, replicate indefinitely, support angiogenesis, avoid apoptosis, and in some cases, metastasize.[2] Molecular profiles obtained by host and tumor DNA sequencing, single nucleotide polymorphism, RNA, and protein microarrays, and methylation screens are

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helping to pinpoint which mutations drive the cancerous phenotype and which are merely passengers on the malignant journey. Notwithstanding the role of individual genes, aggregate molecular profiles provide patient- and tumor-specific information that details the biologic complexity of a particular cancer and can be exploited for its clinical implications, therapeutic insights, and diagnostic benefit.

DETECTION AND MONITORING OF BLADDER CANCER IN THE GENOMIC ERA

Although the treatment for UC has improved over the last several decades, diagnostic techniques have progressed more slowly. Cystoscopy is still considered the best method for diagnosing UC, but it is invasive, uncomfortable, and can only detect approximately 90% of lesions.[3] In addition, when a tumor is discovered and must be biopsied and/or removed, a second procedure is required, transurethral resection of the bladder tumor (TURBT), which requires general anesthesia. Last, the cost of cystoscopy, especially when used to monitor recurrence, is the major reason why per-patient expenses for UC are among the highest for all cancers.[4] The major problem associated with NMIBC is that after initial TURBT, 50% to 70% of patients develop multiple recurrences; 10% to 20% of these will progress to MIBC.[5] This risk of recurrence and progression calls for life-long surveillance. The current standard procedure is to perform cystoscopy and evaluate urine cytology every 3 to 4 months in the first 2 years, twice per year in years 3 to 4, and yearly thereafter.[5]

The burden of this follow-up on the patient, as well as the direct and indirect costs for the patient and society in terms of lost wages, have led to extensive efforts to develop noninvasive urine biomarkers for UC. However, to date, none have demonstrated sufficient specificity and sensitivity to monitor the general population or replace cystoscopy and cytology in monitoring for recurrence.[6] Urine cytology is particularly insensitive for detecting low-grade tumors. However, advances in genomics have clearly demonstrated that DNA alterations offer great promise for detecting primary or secondary bladder cancer.

NMIBC and MIBC are genetically different.[7–10] NMIBC is characterized by a high frequency of mutations in the *FGFR3* oncogene, leading to constitutive activation of the RAS/ MAPK pathway. In MIBC, mutations in the *TP53* gene prevail. In general, mutations in *FGFR3* and *TP53* are mutually exclusive, suggesting that NMIBC and MIBC develop along different oncogenetic pathways. However, these mutations often occur simultaneously in stage pT1 tumors that invade the connective tissue layer underlying the urothelium. Recently, somatic mutations in the *PIK3CA* oncogene, which encodes the catalytic subunit p110α of class-IA PI3 kinase, were described in 13% to 27% of bladder tumors.[11] These mutations often coincided with *FGFR3* mutations. Mutations in the *RAS* oncogenes (*HRAS, KRAS,* and *NRAS*) have also been found in 13% of bladder tumors and in all stages and grades; they are mutually exclusive with *FGFR3* mutations. Given these findings, analyzing urine sediment for genetic mutations may be a promising strategy for noninvasive detection of bladder cancer.

FGFR3

FGFR3 mutations occur in around 50% of both lower and upper urinary tract tumors, clustering in three distinct hotspots in exons 7, 10, and 15.[12] The most common mutations in exon 7 and 10 favor ligand-independent dimerization, transactivation, and signaling.[13–17] Mutations in exon 15 are rare and induce a conformational change in the kinase domain, resulting in ligand-independent receptor activation and signaling, as well as *FGFR3* cellular localization, with aberrant endoplasmic reticulum signaling.[18] *FGFR3* mutations are thought to occur early during urothelial transformation, as they are reported in over 80% of preneoplastic lesions,[13,14] pointing to an overall "benign" effect of *FGFR3* mutation in the bladder.[17,19] *FGFR3*-mutant tumors are more chromosomally stable than their wild-type counterparts. A mutually exclusive relationship between *FGFR3* mutation and overrepresentation of 8q was observed in NMIBC.[20] A recent study found that around 80% of NMIBC and 54% of MIBC have dysregulated *FGFR3* with discordant mutation and protein expression patterns, suggesting a key role for *FGFR3* in both NMIBC and MIBC, either through mutation, overexpression, or both.[21] These discrepancies may reflect differential downstream signaling of wild-type and mutant receptors or the different molecular pathways instigating the development of these tumors. The mechanisms driving *FGFR3* overexpression in UC are largely unknown, although a recent study demonstrated the regulation of *FGFR3* expression in urothelial cells by two microRNAs (miR-99a/100) that are often downregulated in UC, particularly in low-grade and low-stage tumors.[22]

FGFR3 mutations were among the first to be used as urine biomarkers of recurrent disease, especially low-grade disease, which is challenging to detect by urine cytology. van Rhijn et al[23] reported that combined microsatellite and *FGFR3* mutation analysis could detect UC in voided urine. *FGFR3* mutations were found in 44% of urothelial tumors (59 tumors), but were absent in 15 G3 tumors. The sensitivity of microsatellites to detect cancer in voided urine was lower for tumors harboring *FGFR3* mutations (15 out of 21 tumors; 71%) than for *FGFR3* wild-type UC (29 out of 32 tumors; 91%). By including the *FGFR3* mutation, the sensitivity of molecular cytology increased from71%to89% and was superior to the sensitivity of morphologic cytology (25%) for every clinical subdivision. These findings highlighted the potential of molecular biology as an adjunct to cystoscopy and cytology in informing follow-up care.

HRAS

The *HRAS* gene, which codes for p21 Ras (or Ras), a small GT-Pase, was the first identified human oncogene. It was found in the T24/EJ urothelial cell line.[24–26] In the normal urothelium, normal Ras protein diminishes with differentiation, with highest expression in the basal (progenitor) cells.[27] The role of Ras in UC is supported by its ability to transform Simian vacuolating virus 40 (SV40)-immortalized human urothelial cells into invasive transitional-cell carcinomas.[28,29] In addition, in elegant transgenic studies, Ras overexpression has been shown to lead to NMIBC.[30] Ras interacts with Raf, a serine/threonine kinase, which is activated in tumor cells containing enhanced growth signaling pathways in both NMIBC, MIBC, and metastatic disease with subsequent activation of MAPK.[31,32]

P53

The p53 tumor suppressor encoded by the *TP53* gene located on chromosome 17p13.1[33] inhibits phase-specific cell cycle progression (G1-S) through transcriptional activation of $p21^{WAF1/CIP1}$.[34] Most UCs exhibit loss of a single 17p allele. Additional mutations in the remaining allele can inactivate *TP53,* leading to increased nuclear accumulation of the mutant protein, which has a longer half-life than its wild-type counterpart.[35] *TP53* deletion was correlated with grade and stage of UC.[36–41] Invasive carcinoma can also progress from recurrent papillary carcinoma by acquiring additional alterations in *TP53, RB1, PTEN, EGFRs, CCND1, MDM2,* or *E2F.*[42] In addition, oncogenic HRas has been shown to promote the malignant potency of UC cells that have acquired deficiencies of *TP53, RB1,* and *PTEN.*[42] Mutations in the *TP53* gene that result in a truncated protein (or no protein), homozygous deletion of both alleles of the gene, or gene silencing by methylation of the promoters of both alleles cannot be detected by nuclear accumulation of p53 protein,[43,44] thus limiting the sensitivity of immunohistochemistry (IHC) for p53 alterations. Notwithstanding this caveat, overexpression of nuclear p53 protein by IHC has been used as a surrogate marker for detection of mutant p53 in clinical specimens. The expression of p53 has been associated with increased risk of progression of NMIBC or mortality in patients with MIBC, independent of tumor grade, stage, and lymph node status.[33,34,45–56] Interestingly, in a recently reported randomized, prospective trial, this was not borne out in patients treated with cystectomy.[57] In this and other studies, discordance in the identification of p53 as an independent prognostic marker for UC progression, recurrence, mortality, and response to therapy may be a result of patients' genetic and epigenetic status, cohort selection, and technical and statistical variations.[41,58–60]

COMBINING GENOMIC ASSAYS

To develop more sensitive and specific assays, recent studies have simultaneously evaluated *RAS, FGFR3,* and *PIK3CA* in UC.[61] A study of 257 patients with primary bladder tumors found that 64% (164 out of 257) of tumors contained an *FGFR3* mutation, 11% (28) samples were mutant for one of the *RAS* genes, and 24% (61) harbored a *PIK3CA* mutation.[61] Of the 257 primary tumors, 26% overexpressed p53, which is indicative of missense mutations, as noted above. When RAS, *FGFR3,* and *PIK3CA* mutations were calculated with *TP53* mutations, only 27 tumors (11%) were wild-type for all examined genes. In 54 patients who developed one or more recurrences, tissue was available from 184 recurrent tumors, including multifocal recurrences. Using the SNaPshot-based mutation assay, investigators examined these tumors for *FGFR3, PIK3CA,* and *RAS* mutations. The frequency of p53 overexpression was low (6 out of 54) in the primary tumors of this group of patients, consisting mainly of NMIBC tumors. In patients with a wild-type primary tumor, recurrences were mostly wild-type (49 out of 54), whereas five harbored an *FGFR3* mutation. One recurrent tumor contained two different *PIK3CA* mutations. In recurrences, *PIK3CA* mutations in addition to an *FGFR3* mutation were associated with highergrade tumors compared with recurrences harboring an *FGFR3* mutation alone. Importantly, there was 100% consistency in the type of mutation for *RAS* and *PIK3CA* among different tumors in the same patient.

Investigators also developed a methylation assay for specific detection of recurrent NMIBC in voided urine.[62] Microsatellite analysis was also used to detect loss of heterozygosity in voided urine samples.[23] Mutation analysis of *FGFR3, PIK3CA, HRAS, KRAS,* and *NRAS* was recently combined with methylation-specific assays to determine whether this combination outperformed either examination alone.[63] Results were compared with those of urine cytology in a large, retrospective, longitudinal cohort that was part of the European FP7 UROMOL project. A total of 716 voided urine samples from 136 patients with NMIBC (Ta/T1, G½) were collected at TURBT. Patients with a history of carcinoma in situ were excluded from the analysis. Urine was collected at regular follow-up visits immediately before cystoscopy. During follow-up, 552 histologically proven recurrences were detected, including mainly stage Ta (92%), G½ (82%), and solitary tumors (67%). Sensitivity for detecting a recurrent tumor varied between 66% and 68% for the molecular tests after patient stratification based on tumor DNA analysis. A combination of markers increased sensitivity, but decreased the number of patients eligible for a certain test combination. Combining urine cytology with *FGFR3* analysis without stratifying for *FGFR3* status of the incident tumor increased sensitivity from 56% to 76%.

This study highlights the challenge of molecular examination of urine using genomics, and the importance of including all available information (i.e., cytology). However, there is no doubt that next-generation exome sequencing of paired tumor and peripheral blood samples will uncover many more potential biomarkers that could be added to these panels to improve their performance. Such examination was first performed in 2011 in a small set of patients.[9] Initial findings from this cohort were examined in light of findings from an additional 88 patients with bladder cancer[10] and by the The Cancer Genome Atlas (TCGA) consortium.[64] From these contributions, several previously defined mutations were observed (in *TP53, RB1,* and *HRAS),* but novel mutations were also noted, the most common of which was in *UTX,* which was identified in21%of tested individuals. Of note, most of the identified new mutations were related to chromatin remodeling, suggesting a potential new area for bladder cancer research. Mutations in chromatin remodeling genes are commonly found in several other cancer types, suggesting their fundamental contribution to carcinogenesis. Adding to this complexity is a recent study of 537 patients with locally advanced or metastatic UC of the bladder, 74 patients with non-bladder, and 55 patients with nonurothelial bladder cancers profiled using mutation analysis, in situ hybridization, and IHC assays.[65] Compared with nonbladder UC, bladder UC exhibited more frequent expression of abnormal protein (and increased amplification) in HER2, androgen receptor, serum protein acidic and rich in cysteine (SPARC), and topoisomerase 1. These findings suggest that bladder UC has higher levels of actionable biomarkers that may have clinical implications for treatment and diagnostic options.

NEOADJUVANT AND ADJUVANT CHEMOTHERAPY IN MUSCLE-INVASIVE BLADDER CANCER

Approximately 25% of patients with bladder cancer present with a tumor invading the muscle layer of the bladder wall (T2 to T4).[66] MIBC is associated with a high rate of recurrence and poor overall prognosis, despite aggressive local and systemic therapies.

Radical cystectomy is the standard treatment for MIBC, but even with substantial improvements in surgical techniques, mortality remains high because of a high rate of systemic failure. The 5-year mortality rate for patients with MIBC is about 50% to 70%.[67,68] MIBC behaves as a systemic disease, and therefore needs systemic therapy early in the disease process to eradicate micrometastases.[69]

Phase III clinical trials of neoadjuvant cisplatin-based chemotherapy have demonstrated a survival benefit in patients with MIBC,[67,70,71] mainly by pathologic down-staging of muscle-invasive tumors (stage T2 to T4a) to nonmuscle-invasive tumors (< T2).[67,72–74] In the randomized Southwest Oncology group 8710 trial, neoadjuvant methotrexate, vinblastine, doxorubicin, and cisplatin (MVAC) followed by cystectomy demonstrated a 77-month median survival compared with a 46-month median survival with cystectomy alone. In this study, the likelihood of long-term survival was over 85% at 5 years for patients who achieved pathologic down-staging to pT0 either by TURBT alone before cystectomy or by the addition of neoadjuvant MVAC chemotherapy. However, long-term survival was less than 40% at 5 years in patients with residual muscle-invasive tumors (> pT2) at the time of cystectomy in both treatment arms.[67] Patients who do not respond to neoadjuvant chemotherapy have a poor prognosis. However, no data demonstrate a survival benefit for additional chemotherapy in the adjuvant setting after three to four cycles of neoadjuvant cisplatin-based chemotherapy. Large clinical trials are currently in development in this setting.

Data for adjuvant chemotherapy[57,75–77] are less compelling than for neoadjuvant chemotherapy. However, some patients benefit from adjuvant chemotherapy, including those who received up-front radical cystectomy and have extensive tumor invasion of the bladder wall or lymph node involvement. European Organisation for Research and Treatment of Cancer (EORTC) 30994 was a phase III trial of adjuvant versus delayed chemotherapy after cystectomy for patients with pT3 to T4 or node-positive disease.[77] The study randomly assigned 298 patients to one of three adjuvant cisplatin-based chemotherapy regimens (MVAC, high-dose MVAC, or gemcitabine/cisplatin) or observation and chemotherapy at relapse. After a median follow-up of 7 years, 66 out of 141 patients (47%) in the adjuvant chemotherapy arm had died compared with 82 out of 143 (57%) in the observation arm. No significant improvement in overall survival was noted with adjuvant chemotherapy compared with observation (adjusted hazard ratio [HR] 0.78; 95% CI, 0.56 to 1.08; $p = 0.13$). However, adjuvant chemotherapy significantly prolonged progression-free survival compared with observation (HR 0.54; 95% CI, 0.4 to 0.73, $p < 0.0001$), with a 5-year progression-free survival rate of 47.6% (95% CI, 38.8 to 55.9) in the adjuvant chemotherapy arm and 31.8% (95% CI, 24.2 to 39.6) in the observation arm. Although this study did not meet its accrual goal of644 patients and was terminated early, it is the largest randomized adjuvant trial to date. Although the study was limited in power to show a significant improvement in overall survival with adjuvant chemotherapy, it is possible that some subgroups of patients might benefit from adjuvant chemotherapy. Cisplatin-based neoadjuvant chemotherapy remains the standard of care in MIBC. Table 1 summarizes neoadjuvant and adjuvant clinic trials in MIBC.

IMMUNE CHECKPOINT INHIBITION IN SOLID TUMORS

Immune checkpoint inhibition for cancer treatment is an area of growing research. Immune checkpoint pathways regulate T-cell activation to escape antitumor immunity. Immune checkpoint molecules involved in this mechanism include CTLA-4, programmed cell death 1 (PD-1) and its ligands PD-L1 and PD-L2, T-cell immunoglobulin mucin-3, and lymphocyte activation gene-3.[78] Ipilimumab, a monoclonal antibody targeting CTLA-4, a potent immune checkpoint molecule expressed on T cells, demonstrated a survival benefit in a phase III study of patients with metastatic melanoma.[79] PD-1 is an immune inhibitory receptor expressed on several immune-cell subsets, particularly cytotoxic T cells.[80] PD-1 interacts with PD-L1 (B7- H1, CD274), which is expressed on tumor cells and immune cells, including T cells.[81,82] Recent studies have demonstrated that upregulation of PD-L1 is an important mechanism of immune escape in NMIBC.[83–86] Overexpression of PD-L1 in UC correlates with high-grade disease and worse clinical outcome. Anti-PD-1 and anti-PD-L1 have an improved toxicity profile compared with historic data from anti-CTLA-4 clinical trials.[87,88] In September 2014, the U.S. Food and Drug Administration (FDA) granted accelerated approval of pembrolizumab for the treatment of unresectable or metastatic melanoma, and in December 2014 granted accelerated approval to nivolumab for unresectable or metastatic melanoma refractory to standard therapy.

TREATMENT OF METASTATIC BLADDER CANCER

The treatment options for metastatic UC are very limited; however, progress has been made in treating metastatic transitional carcinoma of the urothelial tract with combination chemotherapy. The median survival of 15 to 18 months with either MVAC or gemcitabine/cisplatin is substantially better than the 6 to 9 months with single-agent chemotherapy. In fact, 5% of patients have a complete, sometimes durable, remission.

CLINICAL STUDIES OF PD-1/PD-L1 INHIBITORS IN UROTHELIAL CARCINOMA

Two clinical trials of checkpoint inhibitors have reported preliminary efficacy in advanced/refractory metastatic UC. Remarkable efficacy and safety was seen in a phase I expansion cohort of 67 patients with heavily pretreated metastatic bladder cancer. Patients received 15 mg/kg of MPDL3280A, a human monoclonal antibody to PD-L1 containing an engineered Fcdomain, later revised to a flat dose of 1,200 mg intravenously every 3 weeks.[89] Response rates were reported by PD-L1 positivity status, defined as 5% or higher of tumor-infiltrating immune cells staining for PD-L1 by IHC.[1] In this study, 27% of tumors were IHC 2- or 3-positive, as defined by expression of PD-L1 on tumor-infiltrating immune cells. The overall response rate for all patients by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 was 26%, and was even more remarkable (43%) among patients with PD-$L1^{+}$ tumor-infiltrating cells. Even among patients whose tumor infiltrating immune cells were PD-$L1^{-}$,theresponseratewas11%asmeasured by RECIST v1.1. The median time to first response was 42 days (range, 38 to 85 days). Based on these results, MPDL3280A received breakthrough designation by the FDA in June 2014.

A phase I trial of pembrolizumab/MK-3475, a PD-1 inhibitor, studied 33 patients with advanced UC expressing PD-L1 in at least 1% of tumor cells by IHC. Patients received 10 mg/kg of pembrolizumab every 2 weeks. A response was seen in 7 out of 29 (24%) evaluable patients, and 64% of patients experienced a decrease in target lesions.[90] With a median follow-up of 11 months, six patients have ongoing responses (median duration 16 to 40 weeks; median not reached).

Multiple PD-1/PDL-1 agents are currently being tested alone or in combination in advanced/refractory UC. Many more trials are in development in earlier disease states, testing agents such as MPDL3280A (NCT02302807) in the first-line setting in cisplatin-ineligible patients with metastatic bladder cancer, nivolumab in the maintenance setting after first-line cisplatin-based chemotherapy, and pembrolizumab in patients with NMIBC (NCT02324582).

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KEY POINTS

- Detection of mutations in genes such as *RAS, FGFR3, PIK3CA,* and *TP53,* and methylation pathways in urine sediment are promising noninvasive strategies for diagnosis of bladder cancer.
- The EORTC randomized phase III trial did not show a substantial improvement in overall survival with adjuvant cisplatin-based chemotherapy. Although the trial was limited in power, there was a 22% decrease in the risk of death ($p = 0.13$ trend) and a 55% decrease in the risk of recurrence.
- Cisplatin-based neoadjuvant chemotherapy remains the standard of care in muscle-invasive bladder cancer.
- Immune checkpoint inhibitors have shown efficacy in pretreated patients with metastatic bladder cancer, offering new hope for potential therapeutic strategies in this disease.
- Multiple clinical trials are ongoing and in development in all stages of urothelial carcinoma, testing checkpoint inhibitors alone or in combination with other active agents that enhance the immune system.

TABLE 1.

Summary of Phase III Perioperative Cisplatin-Based Chemotherapy Clinic Trials in Patients with Muscle-Invasive Bladder Cancer

	Stadler (p53)	Cognetti	Paz-Ares	Sternberg	Grossman	MRC/EORTC
Chemotherapy	Adjuvant MVAC × 3	Adjuvant GC × 4	Adjuvant PGC × 4	Adjuvant ddMVAC/ GC/ MVAC × 4	Neoadjuvant MVAC	Neoadjuvant CMV
Patients	T1 and T2 negative LN	T2G3, T3 to T4, N0–2	T3 to T4, N0 to N2	T3 to T4 and/or pT × N1 to N3	T2 to T4aN0	T2 to T4aN0
Design						
α error	5%	5%	5%	5%	5%	5%
Power	90%	80%	80%	80%	80%	90%
Endpoint	Recurrence	OS	OS	OS	OS	OS
	0.5 to 0.3 at 3 years (20%)	50% to > 60% at 2 years (10%)	50% to > 65% at 2 years (15%)	35% to > 42% at 5 years (7%)	35% to > 42% median OS (50%)	50% to > 60% at 2 years (10%)
Hazard Ratio	0.52	0.75	0.77	0.826		
Planned Sample Size	190	610	340	660 (originally 1,344)	298	915
Results						
Patients randomized	114 (499 tested and 272 +p53)	192	142	284	307	976
Years to Accrue	9	6	7	6	11	6
5-Year Recurrence (Observation vs Chemotherapy)	TTR, 0.20; p = 0.62; HR, 0.78	DFS, 42.3% vs. 37.2%; p = 0.70; HR, 1.08; all, 40%	3 years 44% vs. 73%; p < 0.0001; HR, 0.36; all, 54%	PFS, 31.8% vs. 47.6%; p = < 0.0001; HR, 0.54		5-year DFS, 32% vs. 39%; 10-year DFS, 20% vs. 27% p = 0.008; HR, 0.82
5-Year OS (Observation vs. Chemotherapy)	85% (both arms)	53.7% vs. 43.4%; p = 0.24; HR, 1.29; all, 48.5%	31% vs. 60%; p < 0.0009; HR, 0.44; all, 49%	47.7% vs. 53.6%; p = 0.13; HR, 0.78; all, 38.6%	43% vs. 57%; p = 0.06	5-year OS, 43% vs. 49%; 10-year OS, 30% vs. 36%; p = 0.037; HR, 0.84
Median Follow-up	5.4 years	35 months	30 months	7 years	8.7 years	8 years

Abbreviations: CMV, cispiatin/methotrexate/vinbiastine; dd, dose-dense; DFS, disease-free survival; EORTC, European Organisation for Research and Treatment of Cancer; GC, gemcitabine/cisplatin; HR, hazard ratio; MVAC, methotrexate/vinblastine/doxorubicin/cisplatin; OS, overall survival; PFS, progression-free survival; PGC, paclitaxel/gemcitabine/cisplatin; TTR, time to progression.