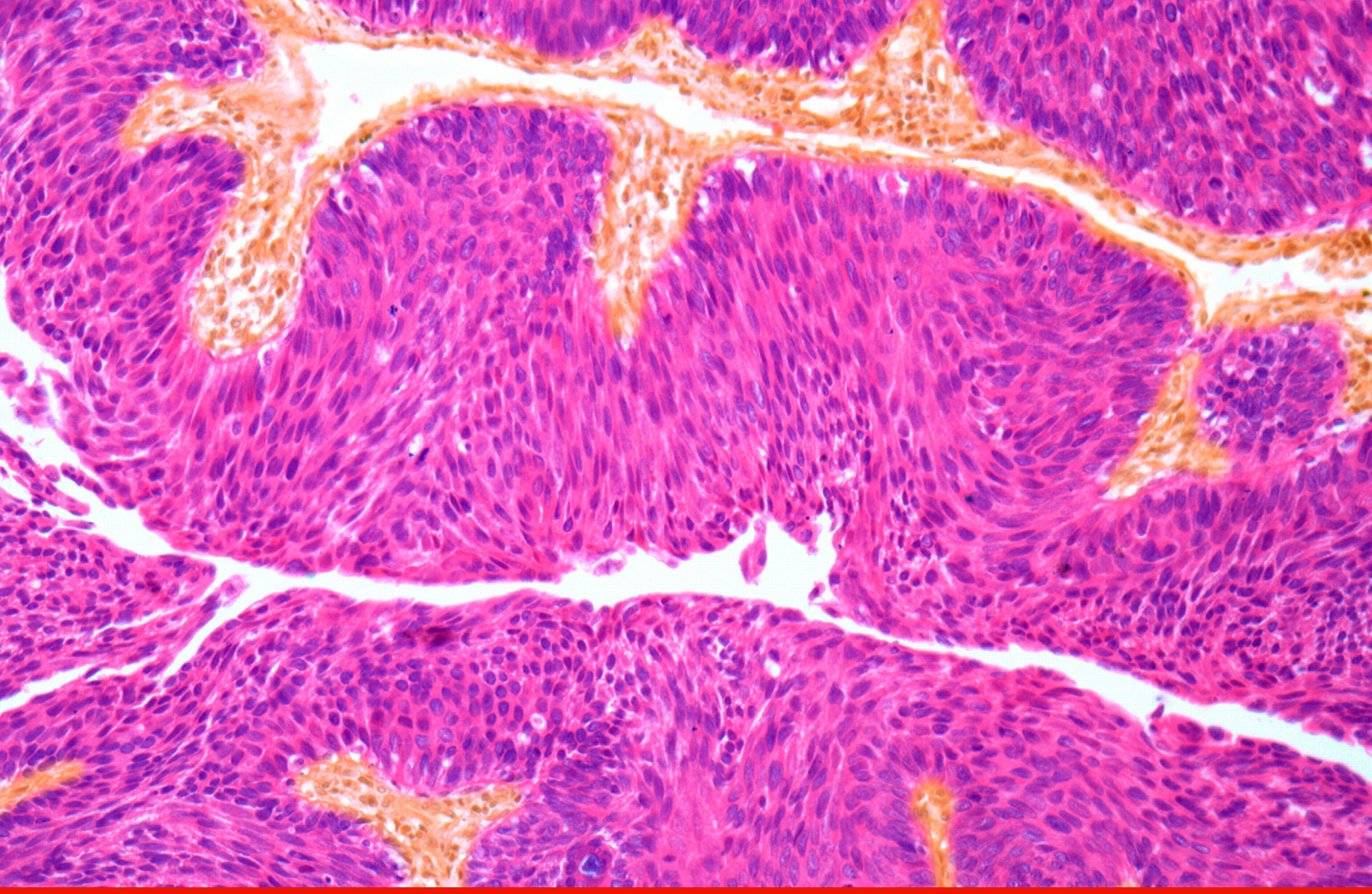

Bladder Cancer Sample Requirements, Testing Methods, and Opportunities

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Precision Medicine

Biomarkers are emerging as useful diagnostic, prognostic, and predictive tools in the management of bladder cancer[1–3]*

Biomarker testing at different stages of disease can inform the management of bladder cancer[1,2]*

Diagnosis
(eg, NMP22)[3]

Monitoring for recurrence
(eg, TERT)[3,4]

Risk stratification and prognosis
(eg, CDKN2A)[5]

Predict likelihood of treatment response
(eg, FGFR3)[1]*

Screen for clinical trials[1]*
(eg, ARID1A)[6]

Guidelines recommend biomarker testing at time of diagnosis of advanced disease[1]*

Diagnostic biomarkers from urine can help clarify equivocal cytology[2]

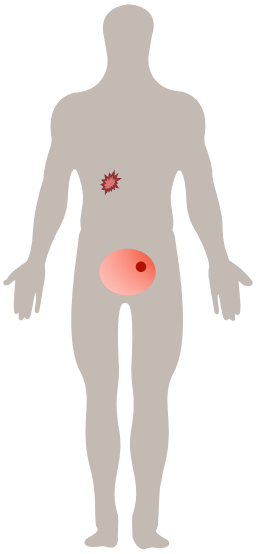
Prognostic biomarkers from tumor tissue or urine can help with risk stratification[7–9]

Predictive biomarkers from tumor tissue can help identify patients for targeted treatments[1,17]*

Bladder cancer biomarkers can be detected in tissue, urine, and blood[2,11]

Samples for biomarker testing

- Cystoscopy with tissue biopsy[7]
- Computed tomography (CT)-guided needle biopsy[12]
- Urine: urinary tumor DNA (utDNA)[13]
- Blood: circulating tumor DNA (ctDNA)[7]



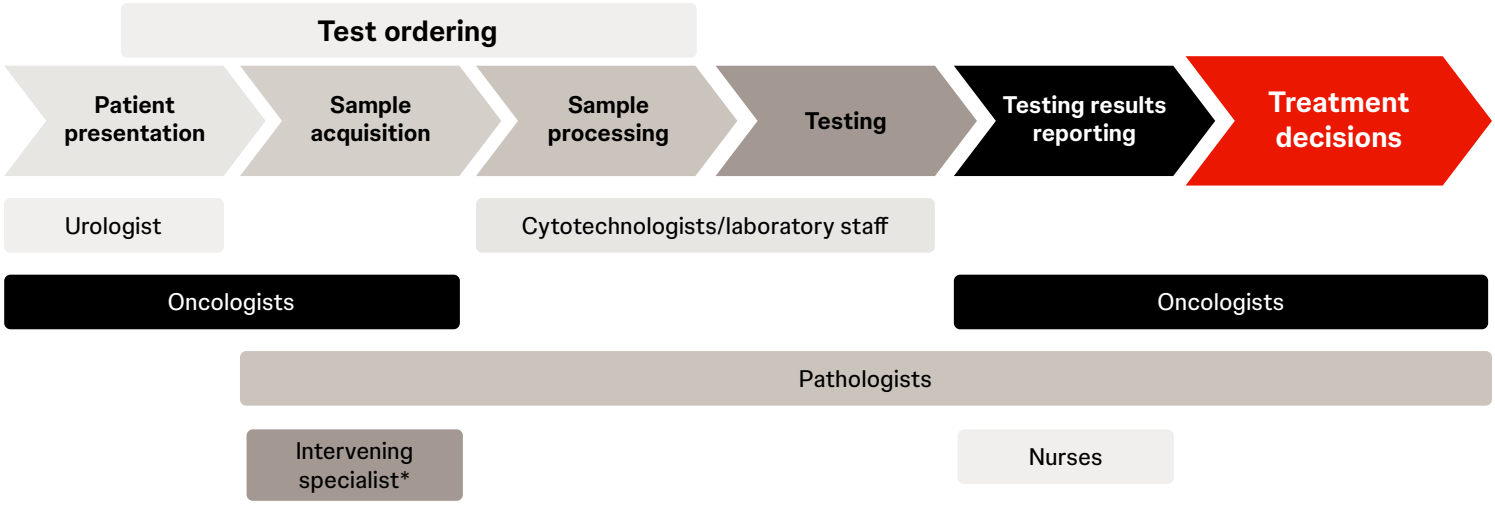
Overview of methods used for assessing biomarkers in patients with bladder cancer

	Single-biomarker tests				Broad-based panel
	Immunohistochemistry (IHC)	**Fluorescence in situ hybridization (FISH)**	**Real-time polymerase chain reaction (RT-PCR)**	**Droplet digital polymerase chain reaction (ddPCR)**	**Next-generation sequencing (NGS)**
Alteration type(s) detected	Protein expression/ overexpression[14]	Amplification, deletions, aneusomies, translocations[15,16]	Mutations, gene fusions, methylation[17,18]	Mutations, genomic rearrangements[19,20]	Single nucleotide variants, copy number variants, insertions-deletions, genomic rearrangements[20–22]

Clinical application	• Identifying flat CIS lesions[23]
	• HER2 and PD-L1 overexpression[14,24]
		• Assess response to intravesical Bacillus Calmette-Guérin (BCG)[2]
		• Clarify equivocal outcomes:
		  • Cytology[2,3]
		  • HER2 IHC[14,25]
		• UroVysion®[16]
		  • Aneuploidy of chromosomes 3, 7, and 17
		  • Loss of 9p21 locus
			• *FGFR3* mutations and fusions[17]
			• Monitoring for recurrence[18]
			• High microsatellite instability (MSI)[26]
				• *FGFR3* and *PIK3CA* hotspot mutations[19]	• Identifying gene mutations and fusions, including *FGFR3*[10,21,22]
					• Tumor mutational burden (TMB)[22]
					• MSI[26]

Communication among multidisciplinary team members is essential for timely and accurate diagnosis[27–29]

Multidisciplinary collaboration is important throughout the biomarker testing process[27–30]



Consultation with pathologists and laboratory personnel prior to sample collection is important when determining protocols for biomarker testing[30]

Biomarker testing can be considered at the time of diagnosis of locally advanced bladder cancer[1†]

Opportunity to optimize

Early consultation with multidisciplinary team members may help ensure that adequate samples are acquired and appropriately prepared for the intended testing procedure[29,30]

*Dependent on cancer stage and procedure.
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Successful biomarker testing involves attention to key steps in the journey

Patient presentation

Determine relevant biomarker test(s)

- Consider biomarker testing for US Food and Drug Administration (FDA)-approved therapies and ongoing clinical trials[1]*
- Consider family history and age at diagnosis for germline testing[1]*
- Order molecular testing at the time of diagnosis to avoid delays[1]*

Sample acquisition

Determine tissue requirements for each test

- Collect enough high-quality tissue for all desired tests[27,29,31]
- Acquire samples from all accessible tumors[27,32]
- Consider alternate sample types: urine, cytology cell block, and blood[7,33]

Sample processing

Determine sample preparation steps for each test

- Maintain spatial orientation of sample when embedding tissue[27]
- Check for inclusion of muscle layer in biopsy[27]
- Use appropriate tissue processing, storage, and preanalytical techniques[29,34]

Opportunity to optimize

Creating standard operating procedures (SOPs) may help determine appropriate testing techniques based on the samples and their required processing[29]

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Biomarkers may improve risk stratification and aid in monitoring high-risk patients[1,7]*

Intertumoral heterogeneity and **variant histology** can complicate risk stratification; **urine biomarkers** are emerging as useful tools to monitor tumor heterogeneity in patients with intact bladders[7,35]

Biomarker testing can improve staging accuracy and may impact the frequency of cystoscopies and treatment options, including bladder-sparing approaches[1,36–38]*

Prognostic biomarkers can be detected in **ctDNA from blood** and **utDNA from urine**[39,40]

Biomarker analysis from biopsy may:

- Assess treatment response to intravesical BCG[2]
- Improve accuracy of risk stratification[7,8]
- Identify appropriate patients for additional treatment[11]
- Reduce unnecessary cystoscopies[41]
- Aid in the evaluation of residual disease, with ctDNA[11,42]

Did you know?

As of 2024, there are currently 6 FDA-approved urinary biomarkers for the diagnosis and surveillance of bladder cancer[3,43]

Biomarkers can also help predict which patients may respond to different treatment strategies[1]*

Important considerations for treatment selection in bladder cancer

- The molecular profile of the tumor(s) contributes to the likelihood of response to different treatments[44]
- A few **targeted treatments** are available **for tumors with specific molecular profiles[1]***
- Matching patients with targeted therapies can lead to improved clinical outcomes[1]*

Biomarker testing for *FGFR3* alterations may help reveal a treatment plan for patients with bladder cancer[1,17,45]*

Oncogenic ***FGFR* alterations,** including point mutations and fusions, may **promote tumorigenesis** by causing constitutive signaling[46–48]

***FGFR3* mutations:**
p.R248C, p.S249C, p.G370C, and p.Y373C[17,49]

***FGFR3* fusions:**
FGFR3-TACC3v1 and FGFR3-TACC3v3[17,49]

Testing considerations

***FGFR3* mutations and fusions can be detected by:**

- RT-PCR[17,50]
  - Requires formalin-fixed paraffin-embedded (FFPE) tumor tissue[17,50]
  - 4- to 5-µm slide with thickness between 100 and 500 mm^2 of total tumor area (can combine from multiple slides)[17]
- NGS[10]
  - In samples from tumor tissue or liquid biopsy[10]

Detection of *FGFR3* alterations is dependent on **sample integrity and the amount of amplifiable DNA** that can be derived from the sample[17]

Testing for *FGFR3* mutations and fusions can be performed on tissue as well as blood[10,17,19]

Biomarker testing rates are improving for bladder cancer, but barriers still exist[51]

NGS testing in advanced bladder cancer has grown over time[51]

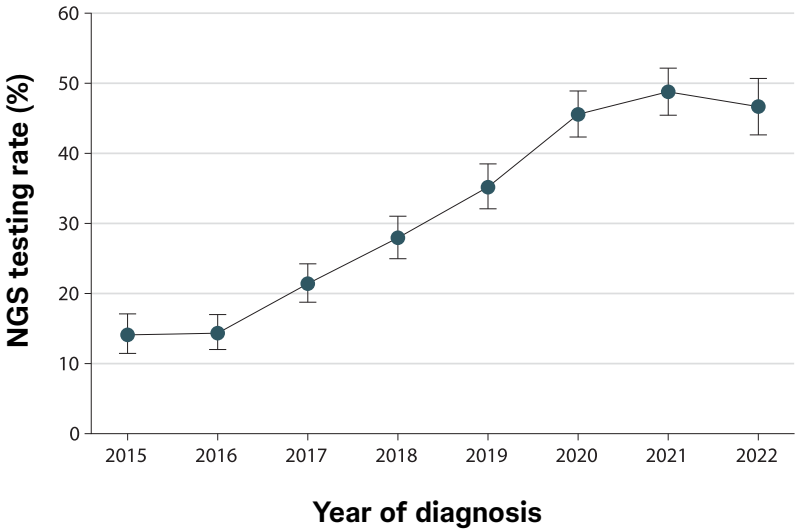

Image adapted from Hage Chehade C, Jo Y, Gebrael G, et al. Trends and disparities in next-generation sequencing in metastatic prostate and urothelial cancers. *JAMA Netw Open*. 2024;7(7):e2423186. doi:10.1001/jamanetworkopen.2024.23186 https://creativecommons.org/licenses/by/4.0/

Barriers to biomarker testing:

Technical limitations

- Low sensitivity and/or specificity for diagnosis of bladder cancer[7]

Cost and accessibility

- Out-of-pocket costs[52]
- Insurance coverage[52]
- Turnaround time (including prior authorizations)[52]

Socioeconomic status (SES) and racial disparities

- Low SES was associated with lower utilization of biomarker testing[51]
- Black patients were less likely than white patients to receive biomarker testing[51]

Potential solutions:

Multi-target diagnostic panels are being developed with increased sensitivity and specificity[53]

Order testing at diagnosis to avoid delays[1]*

Improve awareness about the importance of biomarker testing in underserved communities[51]

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Biomarker tests are being developed to address some of the current challenges in bladder cancer management

Current challenge	Goals	Application of biomarkers
Incomplete TURBT or missed lesions can leave residual disease leading to recurrence/progression[54]	**Reliable detection of residual disease following TURBT or radical cystectomy[54]**	Biomarkers can be used to determine residual disease and inform need for additional treatment[11]
Subjective interpretation and interobserver differences in cytology/cystoscopy[55,56]	**Well-defined, objective criteria for diagnostic consistency[56]**	Biomarker tests can provide objective data that can aid interpretation[55]
Only a subset of patients is eligible for biomarker-informed therapies[57]	**Validate new biomarkers to guide drug development for future treatments[58]**	Multiplexed platforms can efficiently probe for oncogenic mutations to inform future therapies[59]
Repetitive cystoscopies are expensive and burdensome[41,60]	**Develop noninvasive biomarkers that can reliably detect recurrence[61]**	Urine biomarkers are becoming more sensitive/specific[2.59]
Insufficient tissue quality/quantity[62]	**Develop biomarker assays that work on abundant samples like blood or urine[62]**	Detection of actionable biomarkers from ctDNA and utDNA is improving[59,62]
Incorrect staging due to variant subtypes[35]	**Molecularly characterize all variant tumors in a patient[35]**	ctDNA can comprehensively assess heterogeneous tumors[39]

Summary of considerations for effective biomarker testing

Bladder cancer is a complex disease with stage-dependent changes in biomarkers[63]

Different biomarker tests require unique sample preparation and processing requirements that should be considered prior to biopsy[64]

Liquid biopsy in blood and urine is increasingly being studied as a noninvasive way to assess biomarkers in bladder cancer[7]

Precision medicine in bladder cancer involves communication among the multidisciplinary team to optimize patient care[29,30]

ARID1A, AT-rich interactive domain-containing protein 1A; BCG, Bacillus Calmette-Guérin vaccine; CDKN2A, cyclin-dependent kinase inhibitor 2A; CIS, carcinoma in situ; CT, computed tomography; ctDNA, circulating tumor DNA; ddPCR, droplet digital polymerase chain reaction; DNA, deoxyribonucleic acid; FDA, Food and Drug Administration; FFPE, formalin-fixed paraffin-embedded; FGFR, fibroblast growth factor receptor; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; MSI, microsatellite instability; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NMP22, nuclear matrix protein 22; PD-L1, programmed death-ligand 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; RT-PCR, real-time polymerase chain reaction; SES, socioeconomic status; SOP, standard operating procedure; TACC3, transforming acidic coiled-coil-containing protein 3; TERT, telomerase reverse transcriptase; TMB, tumor mutational burden; TURBT, trans urethral resection of bladder tumor; utDNA, urinary tumor DNA.

References: 1. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Bladder Cancer V.1.2025. © National Comprehensive Cancer Network, Inc. 2025. All rights reserved. Accessed March 27, 2025. To view the most recent and complete version of the guideline, go online to NCCN.org. **2.** Holzbeierlein J, Bixler BR, Buckley DI, et al. Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO guideline: 2024 amendment. *J Urol*. 2024;211(4):533–538. **3.** Flores Monar GV, Reynolds T, Gordon M, et al. Molecular markers for bladder cancer screening: an insight into bladder cancer and fda-approved biomarkers. *Int J Mol Sci*. 2023;24(18):14374. doi:10.3390/ijms241814374 **4.** Descotes F, Kara N, Decaussin-Petrucci M, et al. Non-invasive prediction of recurrence in bladder cancer by detecting somatic TERT promoter mutations in urine. *Br J Cancer*. 2017;117(4):583–587. **5.** Verma S, Shankar E, Lin S, et al. 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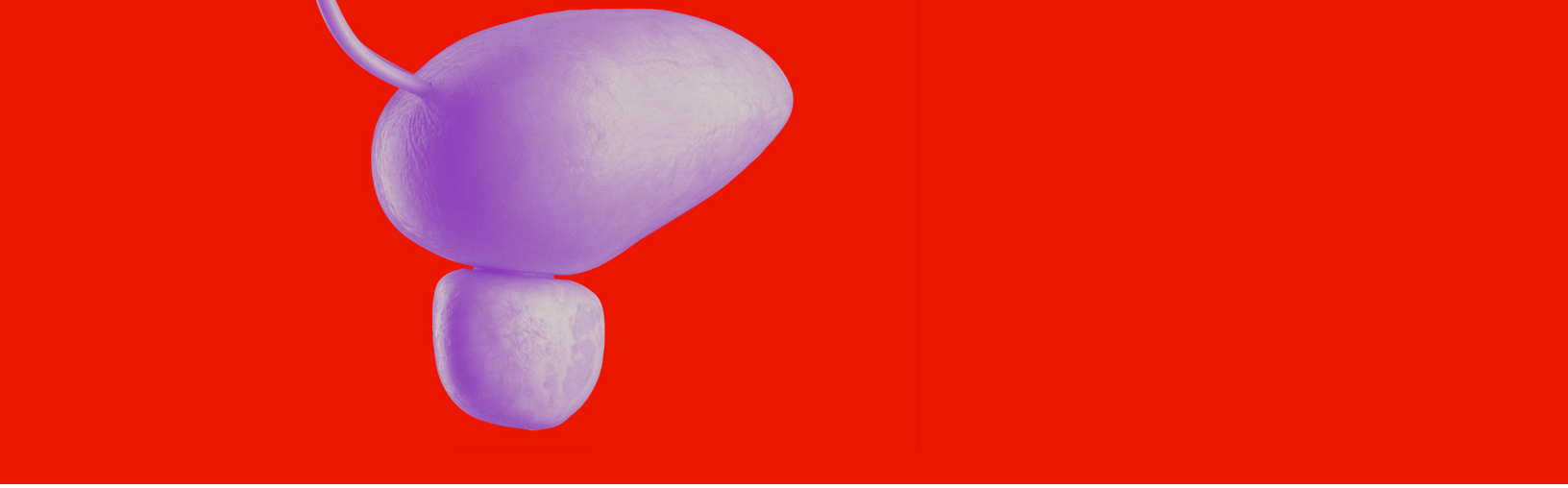

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