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The Diagnostic Network

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Diaceutics
Better Testing, Better Treatment

FGFR3 testing in bladder cancer

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Dr. Michael Hubank

Dr. Hubank has worked in Genomics for over 20 years. Following a PhD at UCL, he worked at the University of Sussex and Yale University before returning to the UCL Institute of Child Health in 2000 to found and run *UCL Genomics*. In 2016 he moved into clinical diagnostics at the Royal Marsden Hospital, London, to lead a translational laboratory focused on the development and clinical application of genomic assays for cancer diagnostics. Since 2018 he has been Scientific Director at the NHS England North Thames Genomic Laboratory Hub.

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Personal conflict of interest disclaimer

In the interest of full transparency, I am disclosing my personal or laboratory support received from Amgen, Astex Pharma, Astra-Zeneca, Bayer, Boehringer Ingelheim, Bristol Myers Squibb, Lilly, Guardant Health, Illumina, Incyte, Janssen, Merck, Novartis, Qiagen, Roche Diagnostics, Servier.

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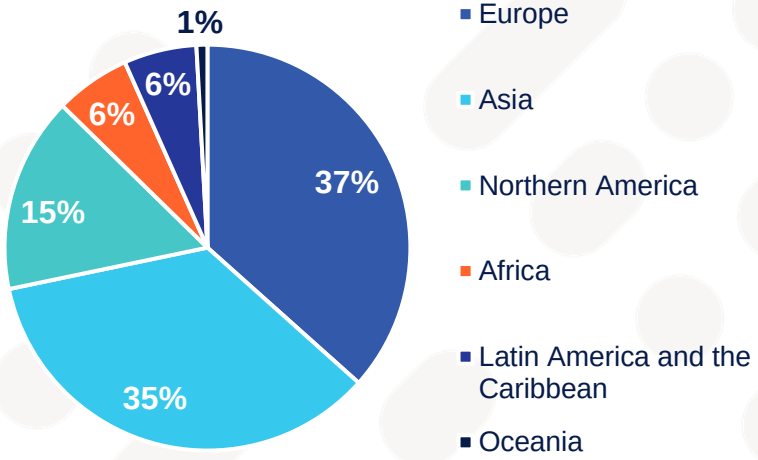
Bladder cancer incidence and mortality

- **Bladder cancer** (BC) is one of the most common urological malignancies worldwide. It is the **9th most common cancer worldwide**, with an estimate of **614,298 new cases in 2022**. It is the **6th most common cancer in men** and the **17th most common cancer in women**.
- In mortality, bladder cancer ranks in 13th position, with **220,596 estimated deaths in 2022.**

- Although **men are more likely to develop bladder cancer**, **women often present with more advanced disease** and have poorer prognosis. This disease can present as **non-muscle-invasive bladder cancer (NMIBC)**, **muscle-invasive bladder cancer (MIBC),** and a metastatic form of the disease.
- The overall survival for bladder cancer patients declines dramatically as the cancer progresses, especially when urothelial cells transition from non-invasive to invasive.

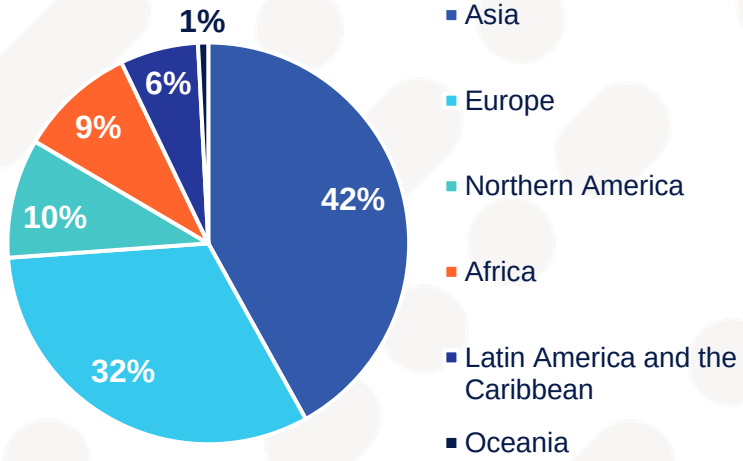
BC incidence, both sexes



BC Incidence		
Rank	Cases	ASR (World)
9	614 298	5.6

BC Mortality		
Rank	Cases	ASR (World)
13	220 596	1.8

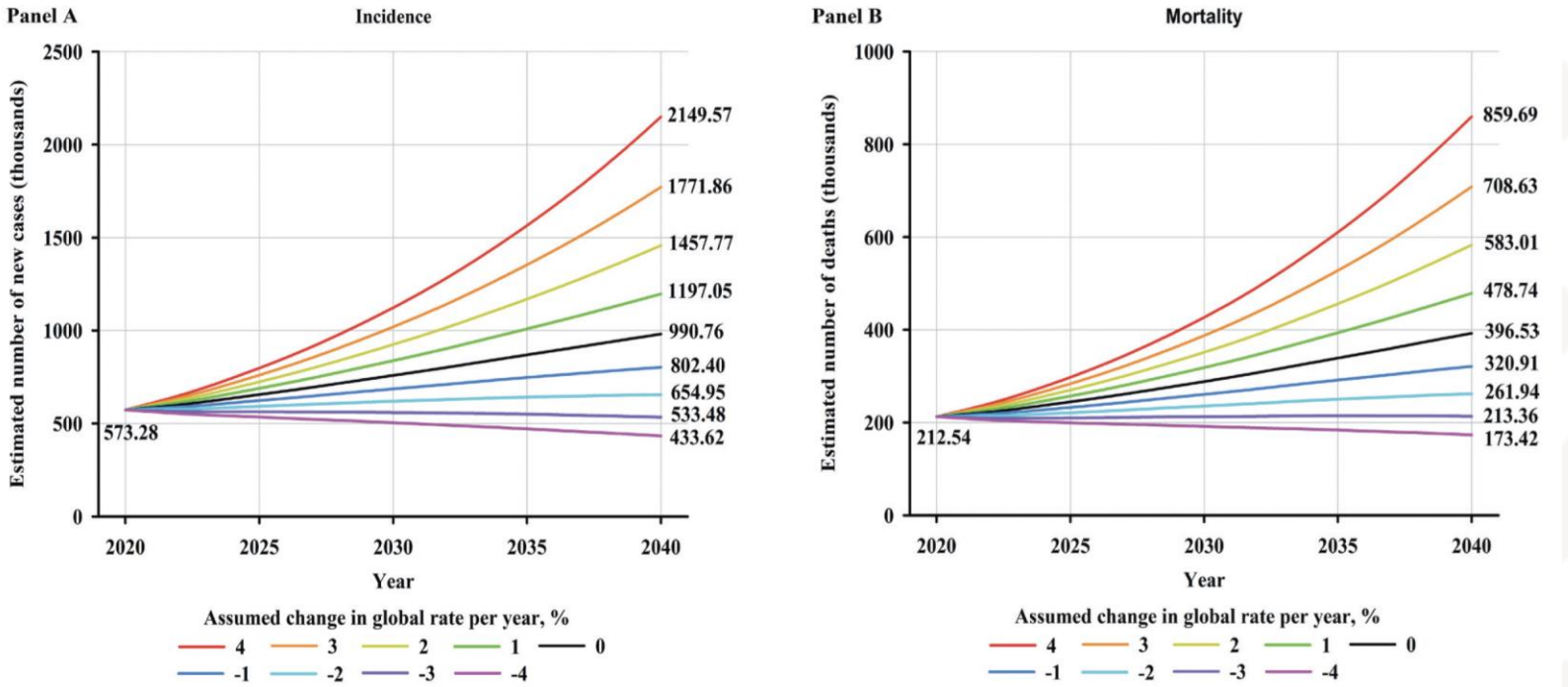
BC mortality, both sexes



WHO GLOBOCAN 2022 data

Bladder cancer predicted incidence and mortality

- According to a study on the global burden of bladder cancer, the number of **new BC cases was estimated to increase worldwide by approximately 72.8%,** from 573,000 in 2020 to 991,000 in 2040. In terms of mortality, the number of **BC deaths was estimated to increase by approximately 86.6%**, from 213,000 in 2020 to 397,000 in 2040.
- By WHO region, the **largest relative increase in new BC cases and deaths will occur in Western Pacific**, with 105.7% more cases and 115.4% more deaths per year by 2040.

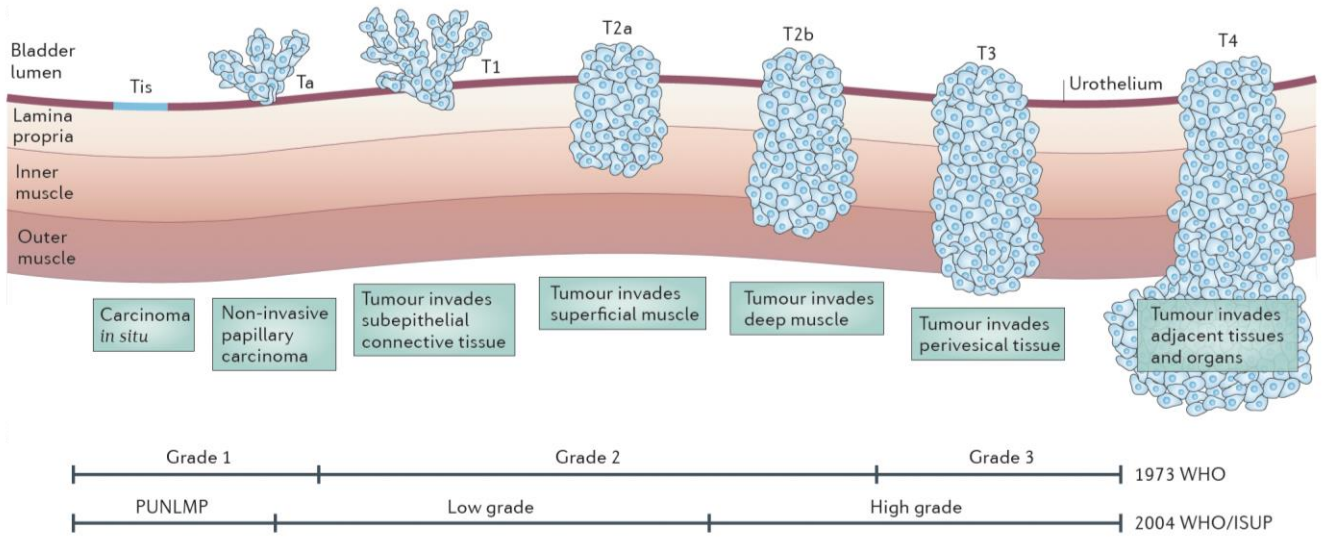


Panel A. Predicted number of new bladder cancer cases for both sexes combined assuming nine scenarios of annual change in global incidence rates between 2020 and 2040. **Panel B.** Predicted number of bladder cancer deaths for both sexes combined assuming nine scenarios of annual change in global mortality rates between 2020 and 2040.

Adapted from et al. J Glob Health 2023;13:04109.

Bladder cancer biology and genomics

- Each stage of the disease is **driven by distinct molecular mechanisms**, with epigenetic dysregulation playing a crucial role in bladder cancer development. Bladder cancer is notably **heterogeneous,** displaying a broad range of clinical and pathological features.
- Gene abnormalities can disrupt the cell cycle, leading to uncontrolled cell proliferation and, ultimately, tumour formation. The **genomic defects associated with bladder cancer are complex, encompassing a wide range of alterations**, from single DNA mutations and gene polymorphisms to partial or complete chromosomal deletions.
- Common **mutations** in bladder cancer include **TP53, PIK3CA, TSC1, FGFR3, HRAS, and HER2**. Common **abnormal expression genes** include **EGFR, Ki67, PD-L1, ERCC1, and BRCA1.**
- **Bladder cancer is comprised of two major groups** based on clinical staging with different clinical outcomes and therapy options:
  - **non-muscle-invasive bladder cancer (NMIBC) – 80% of diagnosed bladder cancer**, stages TIS, Ta and T1
  - **muscle-invasive bladder cancer (MIBC) – 20% of diagnosed bladder cancer,** stages T2a to T4



Bladder cancer grading and staging

- Staging of bladder cancer according to the Tumour–Node–Metastasis (TNM) system.
- Grading according to the 1973 World Health Organization (WHO) and 2004 WHO/ International Society of Urological Pathology (ISUP) criteria.
- The major difference is in the classification of papillary tumours, which are classified as grades 1, 2 and 3 in the older system and as papillary urothelial malignancy of low malignant potential (PUNLMP; equivalent to grade 1), low-grade papillary urothelial carcinoma or high-grade papillary urothelial carcinoma in the WHO/ISUP 2004 classification.

Adapted from Knowles, M., Hurst, C. *Nat Rev Cancer* 15, 25–41 (2015)

Bladder cancer biology and genomics

- **Staging of bladder cancer** is done according to the tumour, lymph node, metastasis (TNM) system. The **UROMOL study** classified **NMIBC into three classes**: class 1, luminal-like signature; class 2, luminal-like, epithelial–mesenchymal transition (EMT) and cancer stem cell signatures; and class 3, basal-like signature. **Six subgroups of MIBC**: luminal papillary (LumP), luminal non-specified (LumNS), luminal unstable (LumU), stroma-rich, basal/squamous (Ba/Sq) and neuroendocrine-like (NE-like).

Non-muscle-invasive bladder cancer (NMIBC)

Subtype	Signatures	Mutations
Class 1 (20%)	• PPARG+ • UPK+ • Early cell cycle genes	**FGFR3**
Class 2 (52%)	• Luminal-like differentiation • PPARG+ • UPK+ • KRT14+ • CIS positive • EMT transcription factors • Cancer stem cell activity • Late cell cycle genes • APOBEC+ signature	• TP53 • ERCC2
Class 3 (27%)	• Basal-like undifferentiation • PPARG– • GATA3+ • KRT5+ • KRT14+ • KRT15+ • CD44+ • RNA-editing signature	**FGFR3**

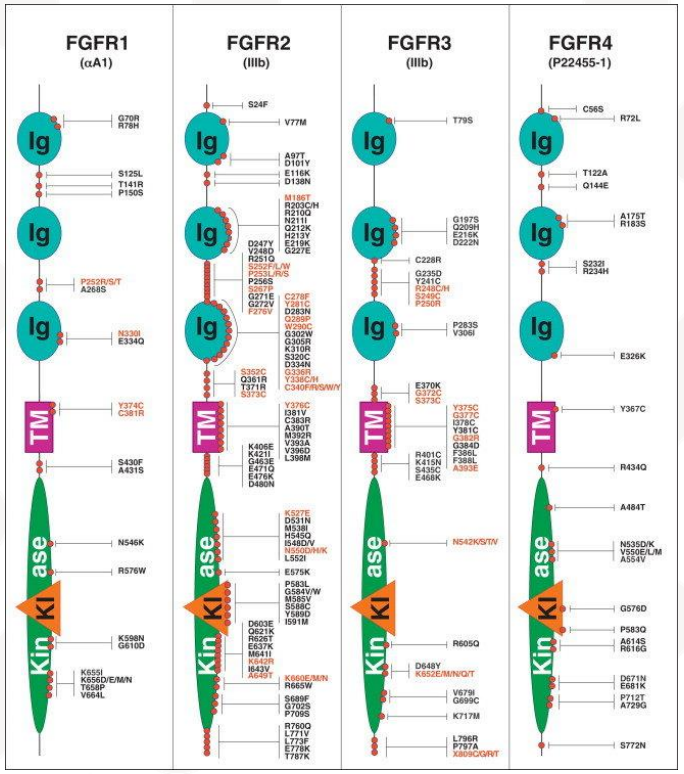
Muscle-invasive bladder cancer (MIBC)

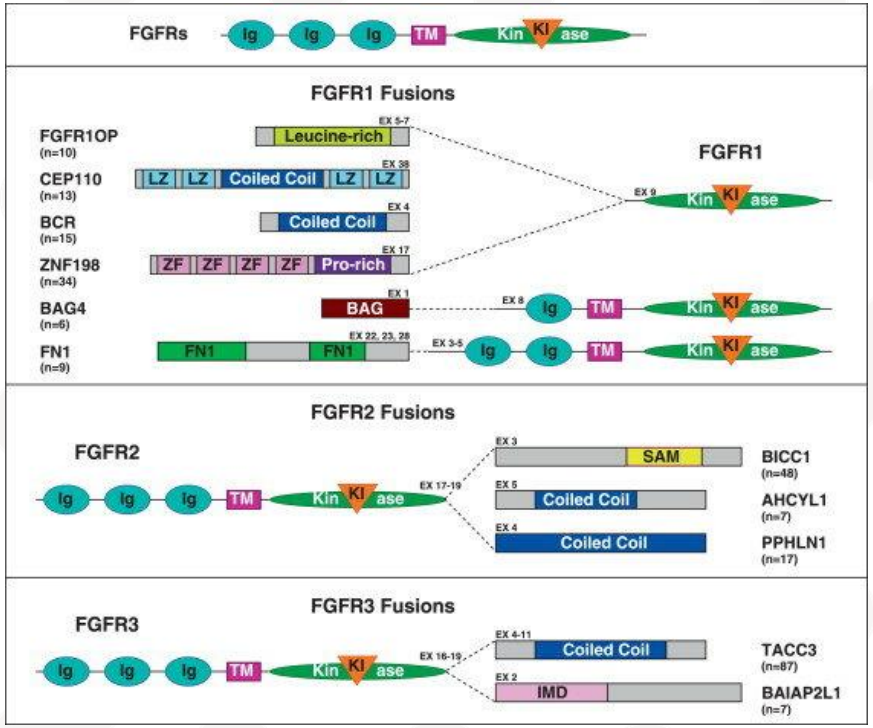
Subtype	Signatures	Mutations
LumP (24%)	• PPARG+ • FGFR3+ • CDKN2A–	• **FGFR3** (40%) • KDM6A (38%)
LumNS (8%)	PPARG+	ELF3 (35%)
LumU (15%)	• PPARG+ • E2F3+ • ERBB2+ • Genomically unstable • Cell cycle positive • APOBEC+ • High TMB	• TP53 (76%) • ERCC2 (22%)
Stromarich (15%)	• Smooth muscle • Fibroblast • Myofiblast gene signatures	–
Ba/Sq (35%)	• Squamous differentiation markers • Fibroblasts and myofibroblast gene signature • EGFR+	• TP53 (61%) • RB1 (25%)
NE-like (3%)	• Neuroendocrine differentiation marker • TP53– • RB1– • Cell cycle positive	• TP53 (94%) • RB1 (39%)

Adapted from Tran, L. *et al. Nat Rev Cancer* 21, 104–121 (2021)

FGFR genomic alterations and their role in cancer

- **Fibroblast growth factors (FGFs) and their receptors (FGFRs)** are involved in many developmental and physiological processes through the **regulation of cell survival and proliferation**. Abnormal FGFR signalling is frequently observed in many types of cancer.
- **Oncogenic FGFR signalling can be deregulated** by various mechanisms, such as **gene amplification, activating mutations and chromosomal translocations**, as well as abnormal FGF ligand-mediated signalling.
- Downstream FGF signalling frequently activates the MAPK–ERK pathway, and in some contexts the PI3K–AKT and Janus kinase–signal transducer and activator of transcription (JAK–STAT) signalling pathways.
- **Multiple FGFR mutations have been identified in human cancer**. These mutations can be present in both developmental syndromes and cancers.





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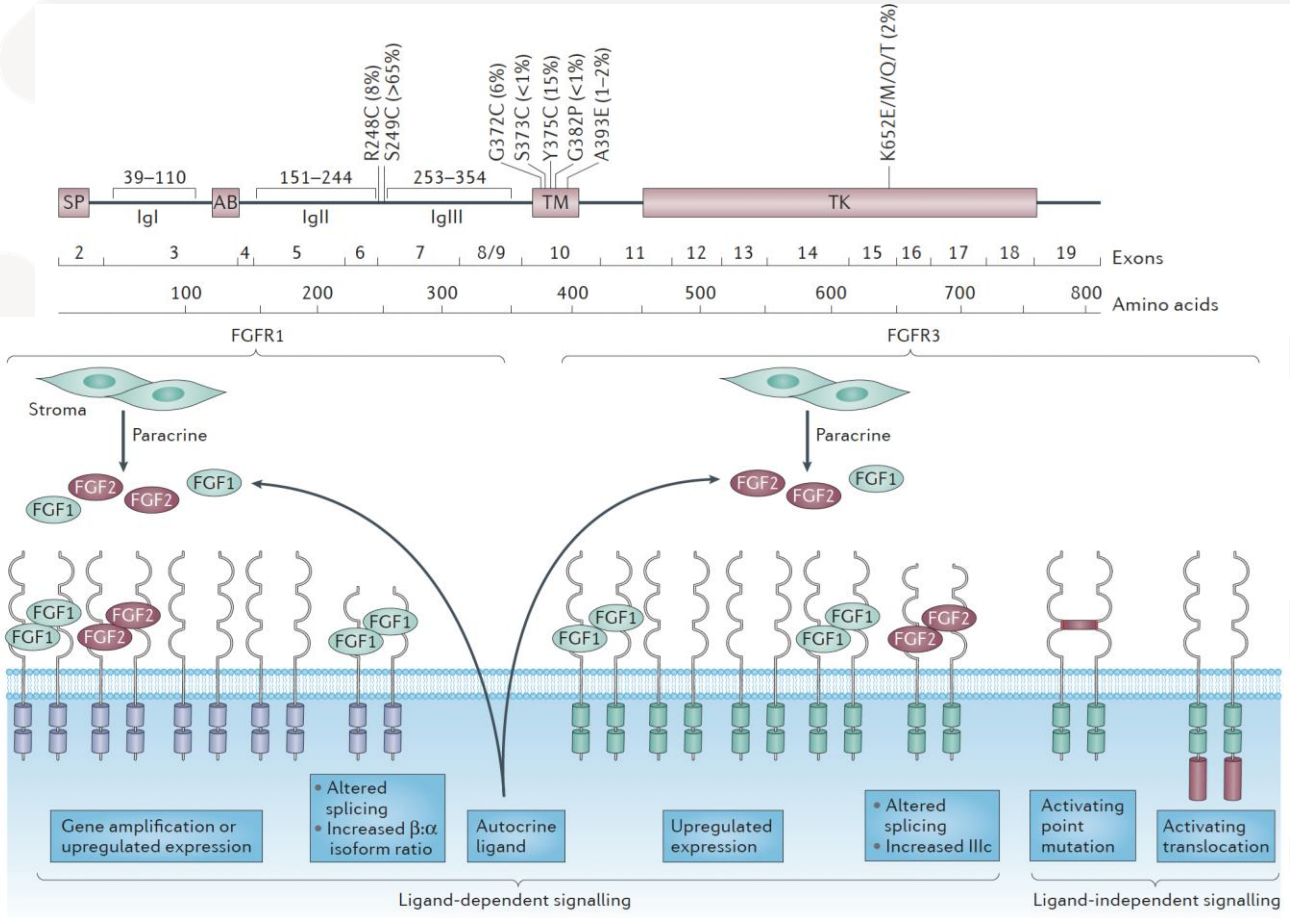
Adapted from Gallo LH. et al. Cytokine Growth Factor Rev. 2015 Aug;26(4):425-49.

FGFR3 and bladder cancer

- **FGFR3 is implicated in the increased risk of developing bladder cancer**. Mutations in **genes encoding FGFR3 were identified as early events in urothelial malignancy.** The exact mechanisms underlying these associations remain unclear, one possibility is that changes in chromatin structure, linked to elevated FGFR3 expression, could raise the probability of mutation and/or amplify the expression and effects of mutated proteins.



FGFR activation in bladder cancer

- A schematic of fibroblast growth factor receptor 3 (FGFR3) protein and corresponding exon positions. Codons showing activating point mutation and relative frequencies as percentage of mutations reported in the literature are indicated. FGFR3 contains the following domains: signal peptide (SP); acid box (AB); immunoglobulin-like domains IgI, IgII and IgIII; transmembrane (TM) region and tyrosine kinase (TK).
- Mechanisms of activation of FGFR1 and FGFR3 in bladder cancer. The receptor-based mechanisms depicted have all been reported in urothelial cancer. There is less information about FGF secretion by urothelial tumour stroma or cancer cells, but FGF-like activity is increased in the urine of patients with bladder tumour.

Adapted from Knowles, M., Hurst, C. *Nat Rev Cancer* 15, 25–41 (2015)

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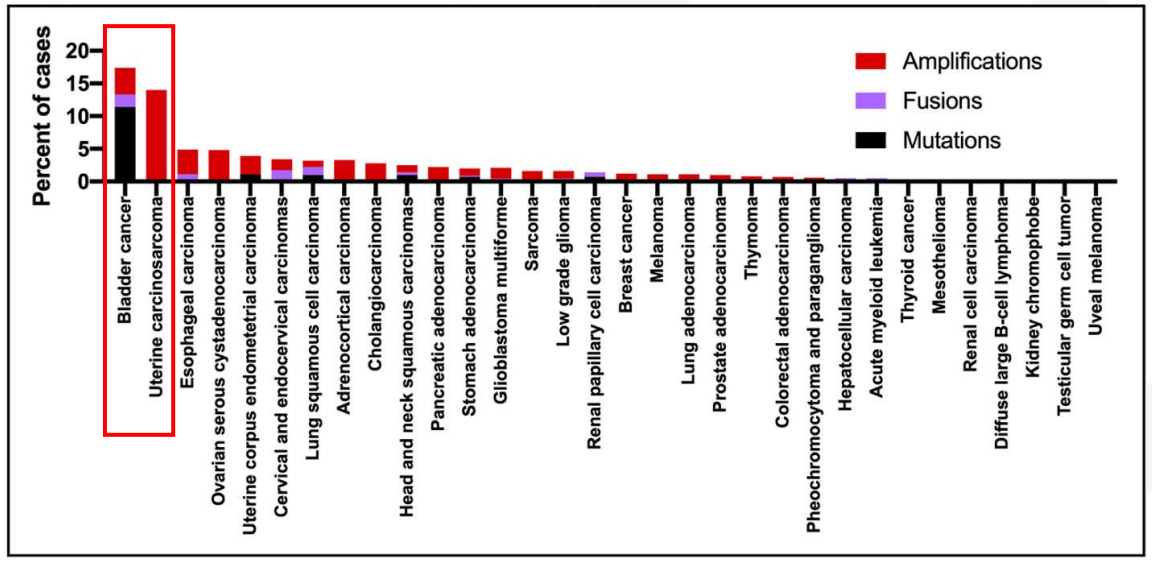
Clinical utility of FGFR3 in bladder cancer

- **Up to 80% of stage Ta tumours have activating point mutations in FGFR3**. In stage T1 tumours and MIBC, FGFR3 mutations are less common (10–20% in tumours stage T2 or above). Luminal tumours (LumP, LumNS and LumU) showed enriched urothelial differentiation, and FGFR3 genetic alterations (mutation, fusion or genomic amplification). MIBC tumours show enhanced activity in pathways involving FGFR3. The prevalence of FGFR3 mutations is approximately 49-84% in localized or non-muscle-invasive bladder cancer (NMIBC) patients, compared to 15-20% in high-risk or muscle-invasive (MIBC) patients.
- Many **bladder cancers**, including those **without FGFR3 point mutations**, show **increased expression of FGFR3.**
- No pro-growth gene in bladder cancer has more activating mutations or amplified expression than the members of the fibroblast growth factor receptor (FGFR) gene family, most notably FGFR3. **Comprehensive molecular testing is key to identifying patients who might benefit from FGFR3-targeted therapies.**



FGFR3 gene alterations by cancer type based on available data from The Cancer Genome Atlas (TCGA) (only recurrent mutations and fusions—those comprising in >1% of mutations/fusions—were included)

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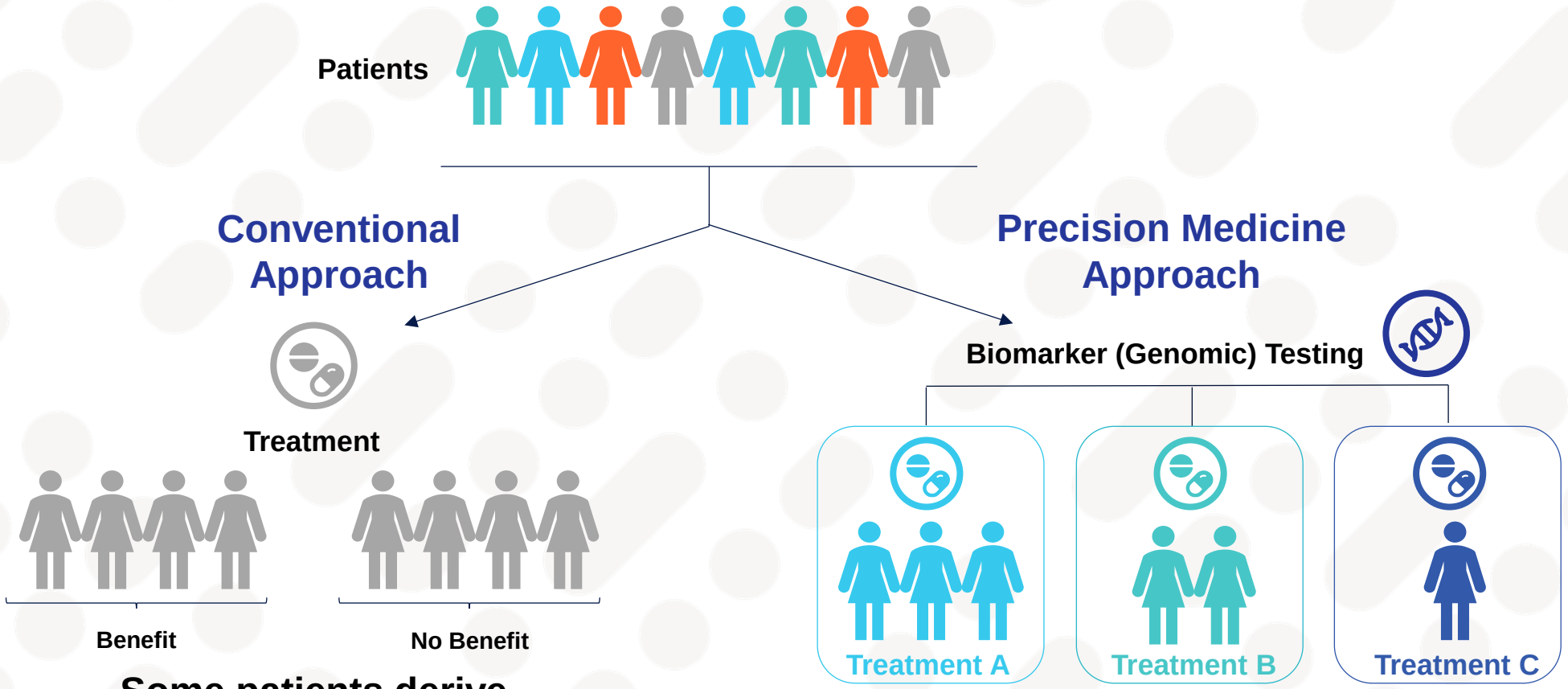
Patient characteristics drive treatment decisions that are most likely to provide benefit



Some patients derive treatment benefit

Genomic tumor profiles allow HCPs to select treatments most likely to benefit the patient

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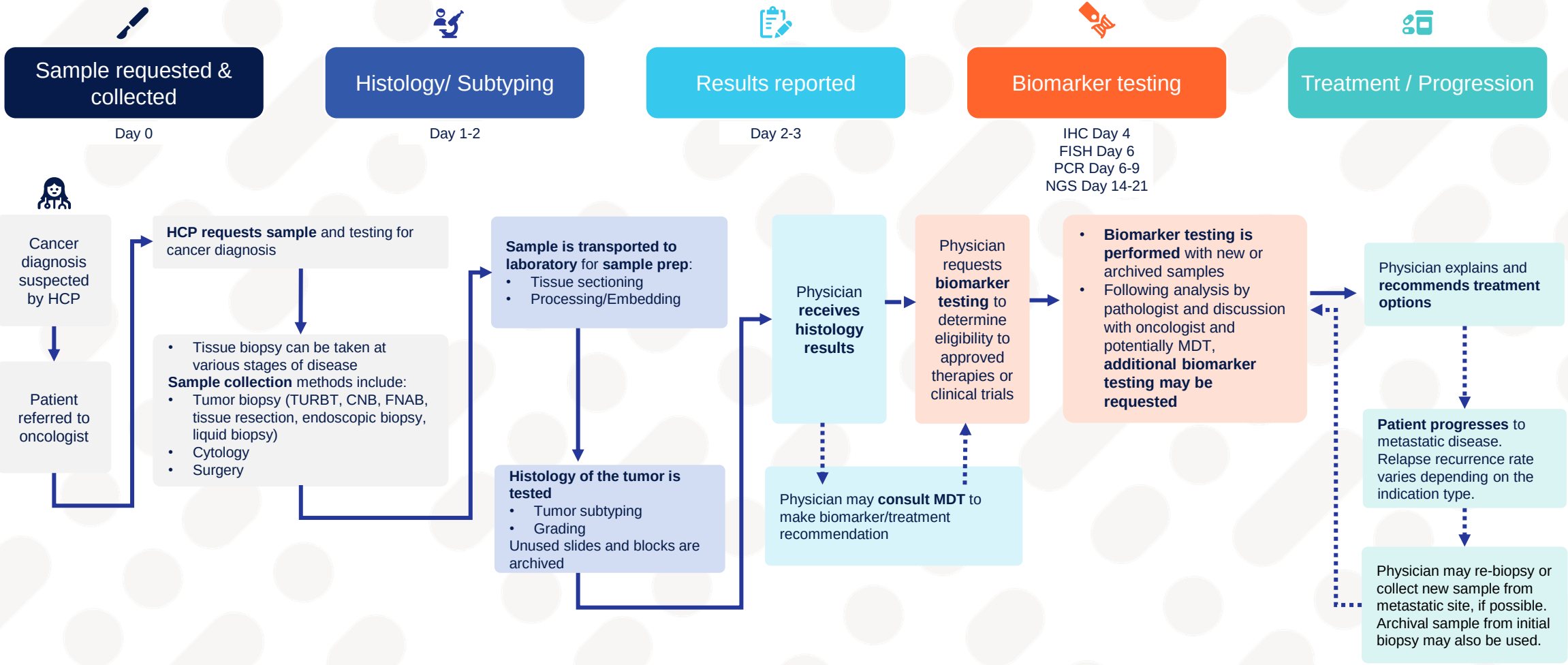
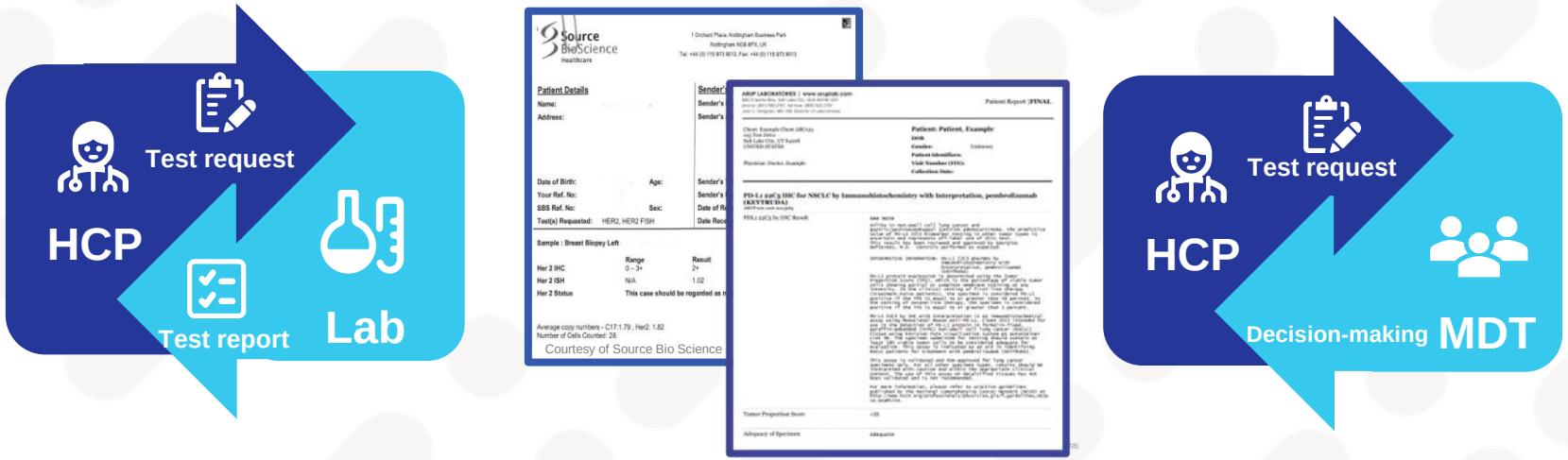
Bladder cancer patient diagnostic journey



MDT collaboration is fundamental to optimizing the diagnostic journey for bladder cancer patients

Results reporting and MDT

Communication flow



Key implications

Communication:

- Test reports are how pathologists communicate findings and occasionally treatment recommendations to HCPs
- Report formats are up to the discretion of laboratories and styles are currently not standardized
- A well-tailored report should be clear, concise, have treatment recommendations and be a clinical resource for HCPs to ensure they are aware of targeted treatment options

MDT:

- Testing and treatment decisions are made by Multi Disciplinary Team meeting (MDT). Reflex protocols may exist for some indications and will not require request or discussion.

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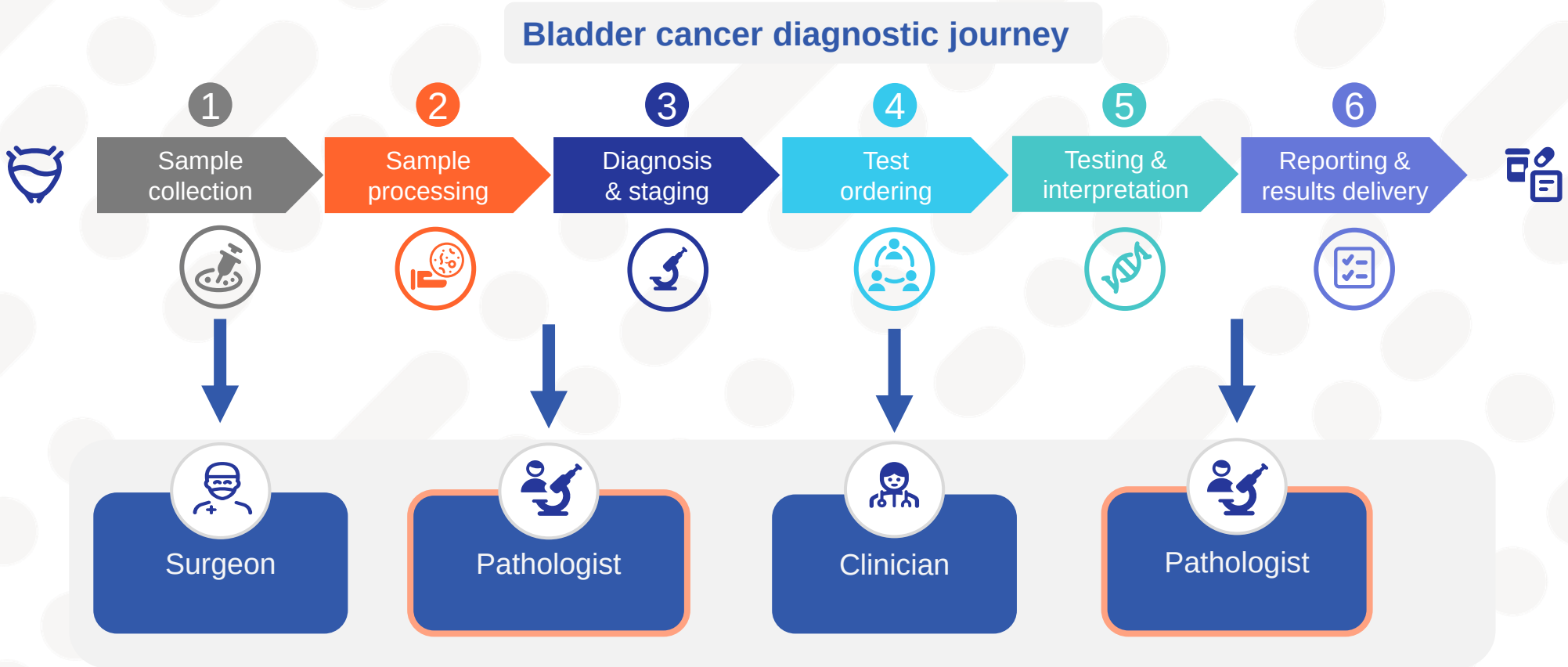
Pathologists are key to ensure optimal FGFR3 testing and timely reporting



Multidisciplinary collaboration is critical to maximizing patient diagnosis to guide treatment decisions

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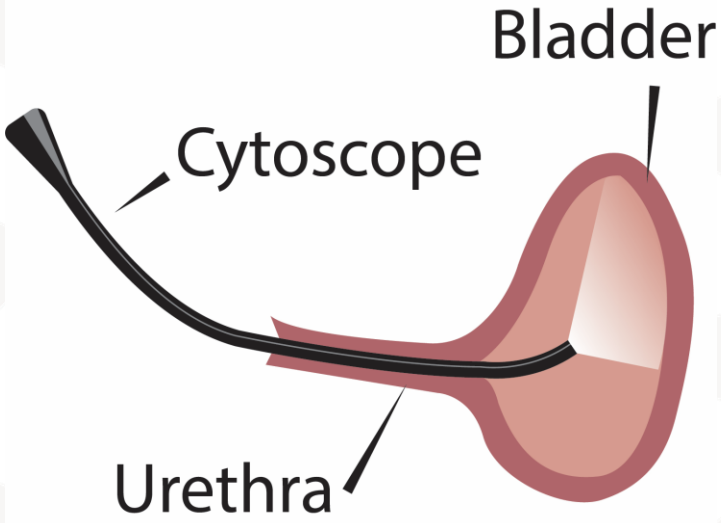
Specimen samples used for the diagnosis of bladder cancer



- **TURBT (Trans urethral resection of bladder cancer)**
  - **TURBT is the main method used to biopsy bladder cancer**
  - When cystoscopy imaging shows bladder abnormalities, a patient should be investigated using TURBT
  - This fulfills two purposes:
    - A sample of the tumor is obtained which is used to **confirm the type and stage** of bladder cancer
    - TURBT is the **first line of treatment,** and may alone be sufficient to remove the tumor and cure disease
- **Urine cytology**
  - This is used to **screen at-risk individuals** rather than symptomatic patients
  - Patients at risk include those previously diagnosed with bladder cancer, exposure to certain chemical and congenital
  - Positive cytology alone is not accurate enough to diagnose bladder cancer and **should be followed up by TURBT**
- **Biopsy from metastatic disease**
  - Where a biopsy of distant metastatic bladder cancer is required, the method of biopsy is dependent on the anatomical location

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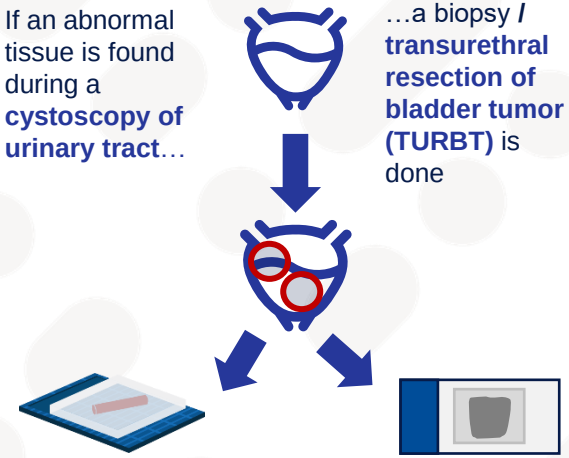
TURBT, FNA and needle biopsies are common tissue acquisition processes for bladder cancer at various stages of disease

Biopsy - tissue acquisition

Primary site – bladder cancer

If an abnormal tissue is found during a **cystoscopy of urinary tract**…

…a biopsy / **transurethral resection of bladder tumor (TURBT)** is done



Abnormal tissue regions from the bladder are collected and processed on FFPE blocks (histologically) or cytological slides

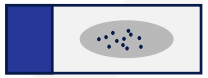
>150 slides. 2-10 histological/ cytological slides are sent for diagnosis and remaining FFPE blocks are archived*

Metastasis – bladder cancer

A biopsy of the **bladder cancer** metastatic site can support establishing a **diagnosis and predict treatment**…

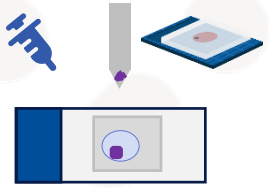
…and **different techniques** are used to collect tumor samples from different sites

Fine Needle Aspirate Cytology (FNAC)



2-3 slides
Cytology smear slides alcohol fixed

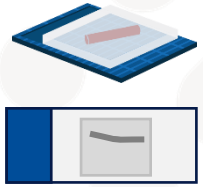
Remaining (if any) aspirate processed to cell block in formalin



5-50 slides*

Needle Biopsy CNB / FNAB and VAB

Tissue cores / fragments in formalin



>150 slides*

- Patients with bladder cancer usually have a histologically or cytologically confirmed urothelial carcinoma in **primary site**, and **radiologically documented metastatic**/unresectable locally advanced disease
- For **treatment purposes**, samples taken at **initial diagnosis** (archived FFPE) or a **new FNAB** (in patients with **metastasis** at lymph nodes, for example), are considered for **biomarker testing**
- **TURBT is a standard method across most markets in bladder cancer.** This is because of the lower invasiveness compared to surgical biopsies, making it more tolerable for sicker patients. As well as the fact it is not just a biopsy method but **also a treatment method.**
- However, **TURBT can result in heterogenous samples**, thus impacting the quality of the tissue available for biomarker testing

Key
Most common sites of metastasis from bladder cancer

- Lymph nodes
- Bones
- Lungs
- Liver
- Peritoneum

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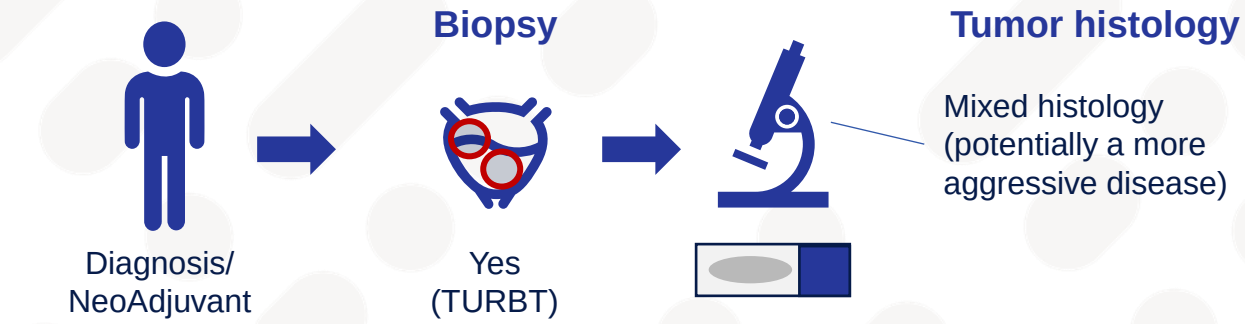
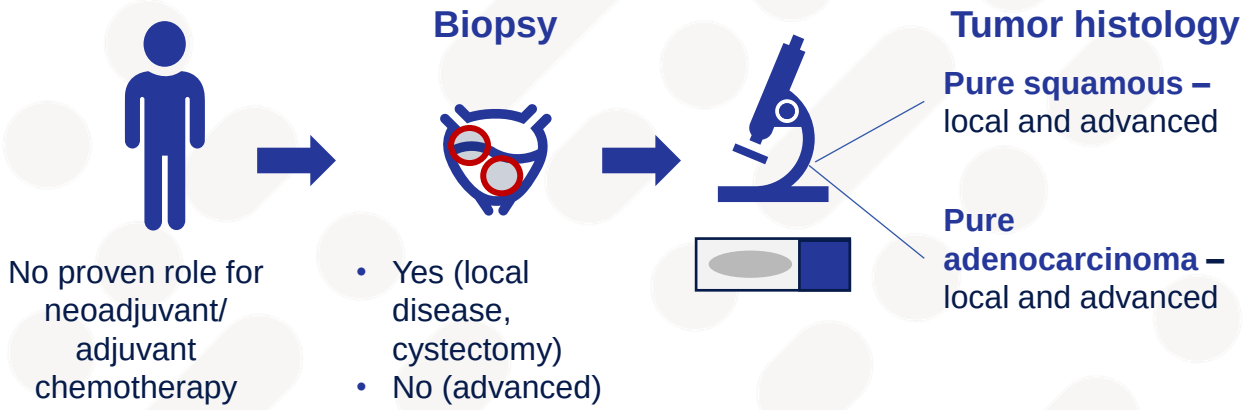
*Number of slides can be variable across laboratories

Mixed histology is managed with neoadjuvant therapy whereas pure squamous/adenocarcinoma have no proven role for Neo/Adj chemotherapy





- UC response to treatment can be variable depending on tumor histology and stage
- The difference of the pathologic staging among some Asian countries may result from the difference of the TNM staging system between AJCC and UICC. **Fortunately, staging does not affect biomarker testing decisions.**
- For local disease, a new sample is collected as surgery is recommended, however, new sample is usually not obtained on a disseminated metastatic disease so **archival tissue from the original biopsy should be used for biomarker testing**

Tissue acquisition in bladder cancer can happen at diagnosis, neoadjuvant setting, 1L and 2L / relapse

- In the bladder cancer **patient journey**, **sample collection** can happen in **three settings**: at diagnosis, neoadjuvant and potentially at 1L/2L (locally advanced disease) settings
- Regarding **sample quality/quantity** for biomarker testing, FFPE archived samples collected via TURBT method **should not be an issue for FGFR3 or additional tests**

Steps of patient journey/setting	Sample type	Biopsy methods	Biomarkers¥ requested	Methods for testing
Diagnosis	New	TURBT, Cytology	**FGFR2, FGFR3**	RT Real-Time PCR
Neoadjuvant	New	TURBT	Not at this stage	
Adjuvant	*No collection*			
1L	New/Archived	TURBT, Cystectomy	PD-L1 **FGFR2, FGFR3**	IHC Real-Time PCR
2L/Relapse/ Metastatic	New/Archived	TURBT, Cystectomy	PD-L1 **FGFR2, FGFR3**	IHC RT Real-Time PCR

¥ Highlighted only predictive/CDx biomarkers

Molecular testing should include analysis of FGFR2 and FGFR3 genetic alterations for targeted treatment. Ideally, molecular testing is recommended at diagnosis of advanced bladder cancer (newly collected samples). However, in metastatic patients with no sample collection feasibility, testing is done in archived samples collected at diagnosis

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Regardless of the biopsy type, tissue should be available for testing from new or archived tissue

Primary/locally advanced tumor

A typical bladder **TURBT** sample will give around **1500µm** of tissue

- 1 x 10µm H+E
- 8 x 10µm Ab
- 2 x 10µm controls

= 110µm for tests + 20µm wastage

Total = 120µm

- 25 x 10µm FGFR2/FGFR3 test
- 1 x 10µm H+E
- 2 x 10µm PD-L1

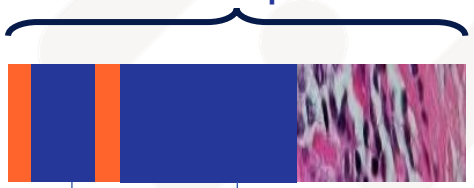
= 280µm for tests + 20µm wastage

Total = 300µm

- Total tissue required for bladder cancer diagnosis and biomarkers tests = **430µm**
- Remaining tissue for additional tests = **1070µm**

*Metastatic tumor

A typical **CNB** sample will give around **600µm** of tissue



- 1 x 10µm H+E
- 8 x 10µm Ab
- 2 x 10µm controls

= 110µm for tests + 20µm wastage

Total = 120µm

- 25 x 10µm FGFR2/FGFR3 test
- 1 x 10µm H+E
- 2 x 10µm PD-L1

= 280µm for tests + 20µm wastage

Total = 300µm

- Total tissue required for bladder cancer diagnosis and biomarkers tests = **430µm**
- Remaining tissue for additional tests = **170µm**

- TURBT is the preferred biopsy type for primary/locally advanced bladder tumors
  - Histology, H&E consumes 130µm
  - FGFR2/3, PD-L1 biomarker testing consumes 300µm
  - **1070µm tissue remains** to be archived and used for future testing
- In the metastatic setting, new and/or archived sample is used for further biomarker testing. Depending on the metastatic site, a new biopsy of the metastatic tissue may not be possible, however a re-biopsy of the bladder is an option if more tissue is needed

20µm Block trimming waste

µm Tissue amount used for tests

***Metastatic tumor**

Where a biopsy of distant metastatic cancer is required, the method of biopsy is dependent on the anatomical location. **CNB** is one of the biopsy methods used for lung cancer, the most common metastatic site across tumors inside this report.

Technology preference is initially informed by the nature of the biomarker target, tissue, performance criteria of different technologies as well as clinical accessibility of testing platforms

Testing technologies	FISH	PCR	NGS	IHC	Flow Cytometry
Target analytes	Chromosome	Molecular; DNA, RNA		Protein	Cell
Test lab	Cytogenetics	Molecular Genetics		Histopathology	Hematology
Variant type	Amplification Deletions Fusions	Genomic alterations: Single nucleotide variants (SNVs) Insertions and deletions (InDels) Copy number variants (CNVs) Fusions		Protein expression	Antigen expression

Next generation sequencing (NGS)

1. DNA/RNA EXTRACTION
From various sample types

2. PCR REACTION
Amplifies many regions of interest simultaneously

3. MASSIVELY PARALLEL SEQUENCING
Enrichment using hybrid capture versus amplicon based

4. SEQUENCE ANALYSIS
Complex bioinformatic analysis

Instrument reads massive amounts of DNA fragments simultaneously

Computer software aligns, filters noise and compares sequence to reference genome

Advantages	Challenges
• Multiple targets simultaneously analyzed • Availability of commercial kits that cover gene fusions • RNA based NGS can be used to detect gene fusions • Decreased cost/gene	• Specificity and detection capabilities vary depending on enrichment method (hybrid capture versus amplicon based) • Long turnaround time (5–20 days) • Requires complex bioinformatics

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Fluorescence in-situ hybridization (FISH)

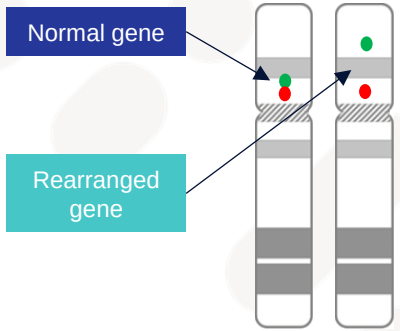
1. TISSUE FIXED AND EMBEDDED WITHIN PARAFFIN BLOCK

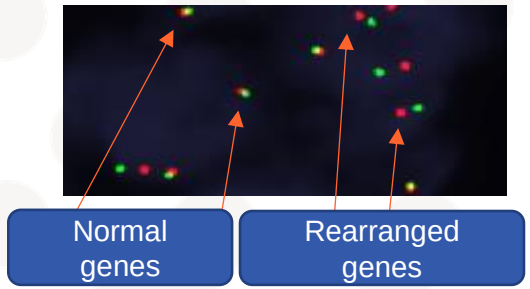
2. SECTIONS PLACED ON A MICROSCOPE SLIDE

3. DNA PROBE HYBRIDIZED TO THE CELLS
overnight incubation

4. ANALYSIS USING A FLUORESCENCE MICROSCOPE

- All normal cells have **2 copies** of any particular gene; each located on one of a pair of chromosomes
- Two probes for a gene can be labelled with fluorescent markers each having a unique color for the 5’ and 3’ ends of the gene
- Each gene is therefore represented as two co-located green and red spots
- Rearrangement of a gene is indicated when the green and red dots are split apart (other FISH strategies can also be employed)





Advantages	Challenges
• Simple process • Genes with multiple fusion partners can be identified in a single test • Considered the gold standard in some conditions • Used for copy number variations and fusions	• Labour intensive and time consuming – skilled • Specialized equipment required • Cannot detect single nucleotide mutations types

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Real Time/Reverse Transcription-PCR

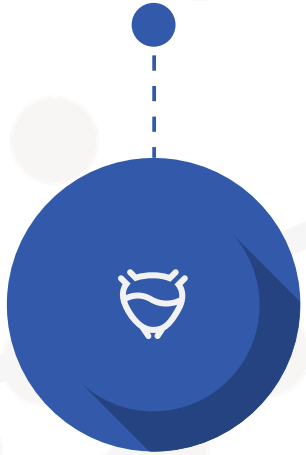
1. DNA/RNA EXTRACTION AND cDNA SYNTHESIS
From various sample types and platforms

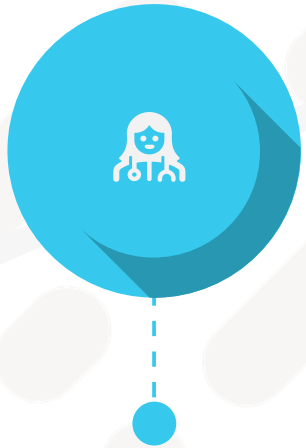
2. RT-PCR REACTION
Counts normal and mutant copies amplified in real time (i.e. as the PCR reaction takes place)

Advantages	Challenges
• Rapid and sensitive test • Assay can be multiplexed to cover a range of mutations within a single reaction • Well established within molecular genetic laboratories for quantitatively monitoring therapy response	• Assay probes have to be designed for each specific fusion combination • Potential presence of PCR inhibitors in the biologic sample • cDNA RT-PCR is susceptible to false positives due to DNA contamination

Key messages

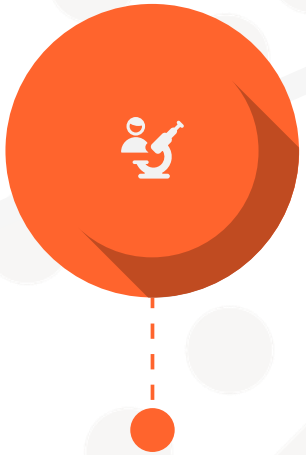
FGFR3 alterations play a pivotal role in the development and progression of bladder cancer





The **bladder cancer diagnostic journey** is complex, and MDT collaboration is key

Identifying **patients who will benefit most from FGFR3-targeted therapies** requires reliable and comprehensive molecular testing



The **choice of test** may depend on the clinical context, available resources, and specific FGFR3 alterations of interest

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