RETRACTED: Animal Models in Bladder Cancer

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Abstract: Background: Bladder cancer (urothelial cancer of the bladder) is the most common malignancy affecting the urinary system with an increasing incidence and mortality. Mouse models of bladder cancer should possess a high value of reproducibility, predictability, and translatability to allow mechanistic, chemo-preventive, and therapeutic studies that can be furthered into human clinical trials. Objectives: To provide an overview and resources on the origin, molecular and pathological characteristics of commonly used animal models in bladder cancer. Methods: A PubMed and Web of Science search was performed for relevant articles published between 1980 and 2021 using words such as: "bladder" and/or "urothelial carcinoma" and animal models. Animal models of bladder cancer can be categorized as autochthonous (spontaneous) and non-autochthonous (transplantable). The first are either chemically induced models or genetically engineered models. The transplantable models can be further subclassified as syngeneic (murine bladder cancer cells implanted into immunocompetent or transgenic mice) and xenografts (human bladder cancer cells implanted into immune-deficient mice). These models can be further divided—based on the site of the tumor—as orthotopic (tumor growth occurs within the bladder) and heterotopic (tumor growth occurs outside of the bladder).

Keywords: animal models; bladder cancer; urothelial cancer; xenografts

1. Introduction

The most common malignancy of the urinary tract and the second most common malignancy of the urogenital tract following prostate cancer in the United States is bladder cancer [1]. Bladder cancer is now the fourth most common among men and the ninth most common in women. Approximately 90% of affected patients are over 55 years of age, with a mean age at diagnosis of 73 years. Men are three to four times more likely than women to develop the disease, with a lifetime risk of one in 26 for men and one in 88 for women. Bladder cancer affects whites about twice as often as blacks or Hispanics but is more likely to be diagnosed at an advanced stage in patients of color. As the incidence of the disease has decreased, mortality from bladder cancer has decreased in women but remains unchanged in men [2].

The primary risk factors for bladder cancer contain environmental and occupational exposures to chemical carcinogens [3] such as tobacco smoke/metabolites, aromatic hydrocarbons, house paints, fungicides, plastics, and heavy metals [4–6]. A family history of bladder cancer is linked to a two-fold higher risk, however, bladder cancer-affected families are not common and no high-penetrance genes have been identified [7–9]. N-nitroso

compounds (NOCs) used in chemically preserved food products are associated with the risk of bladder cancer (Figure 1) [10].

Aristolochic acid (AA) is a naturally occurring compound that can specifically induce acute nephrotoxicity as well as bladder cancer [11].
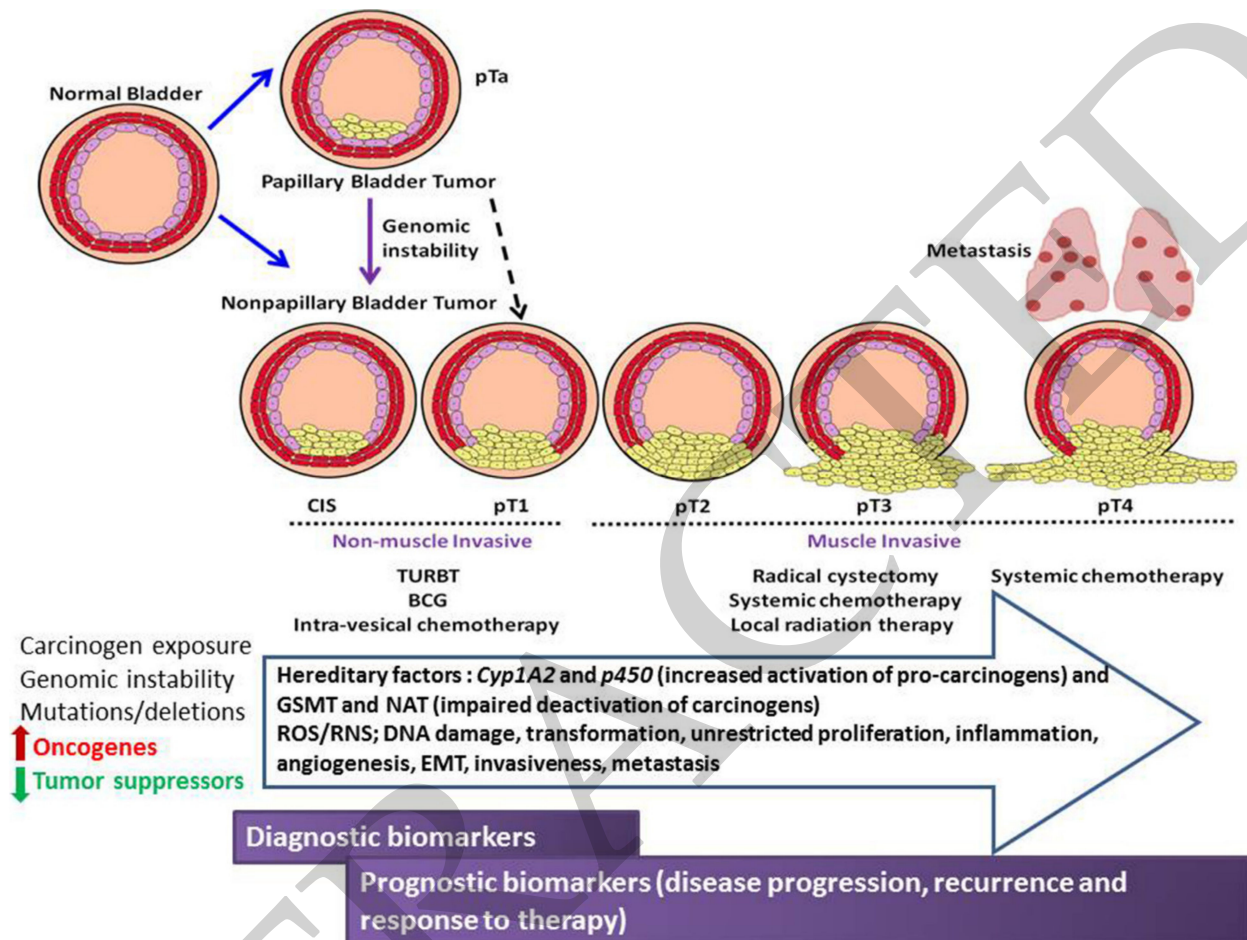

Figure 1. Schematic illustration of the pathological stages of urothelial cancer of the bladder, therapeutic intervention, environmental, genetic, and molecular influences of urothelial carcinogenesis, progression, and metastasis. Opportunities for diagnostic/prognostic biomarker discovery are shown compared to disease stage. (Adapted from© Bincy Anu John1 and Neveen Said, Insights from animal models of bladder cancer: recent advances, challenges, and opportunities, Oncotarget, 2017, Vol. 8, (No. 34) [12]). CIS—carcinoma in situ; TURBT—transurethral resection of bladder tumor; BCG—Bacillus Calmette–Guérin; GSMT—glycine sarcosine methyltransferase; NAT—N-acetyltransferase; ROS/RNS—Reactive oxygen and nitrogen species; DNA—desoxyribonucleic acid; EMT—epithelial to mesenchymal transition.

Bladder cancer typically grows from the urothelium, the well-differentiated transitional epithelium that lines the urinary bladder. The majority of bladder cancers diagnosed in developed countries are of transitional cell histology and are known as urothelial carcinoma (UC). Bladder cancer develops via two clinically and pathologically distinct routes: papillary and non-papillary forms of disease [9,10]. These tumors may be multifocal and may appear repeatedly after local excision, but typically do not invade the bladder wall or metastasize—periodic cystoscopy is the mainstay of non-muscle invasive bladder cancer (NMIBC) management, as high grade (HG) tumors have progression rates reported from 15–40% and even low grade (LG) tumors have a mean recurrence rate of 50% [13].

Approximately 75–80% of urinary bladder tumors are superficial papillary lesions known as non-muscle-invasive urothelial cancer (NMIUC and are referred to as low-grade intra-urothelial neoplasia. Low-grade lesions are associated with molecular aberrations in RAS, fibroblast growth factor receptor 3 (FGFR3) oncogenes, and 9q deletions [14].

The bladder is composed of a specialized epithelium, called the urothelium, which is surrounded by the lamina propria and a thick layer of smooth muscle (the detrusor muscle or muscularis propria), which forms the bladder wall [15,16]. The urothelium includes three cell types: basal cells, which are relatively small cuboidal cells expressing p63 and high molecular weight cytokeratins, such as 5 (KRT5) and 14 (KRT14); intermediate cells, which also express p63 and high molecular weight cytokeratins, although at lower levels than the basal layer; and superficial cells, also called "umbrella cells", which express uroplakin proteins and low molecular weight cytokeratins 18 (CK18) and 20 (CK20) [17–21]. The superficial cells have polarized membranes that are insoluble, and specialized structures on their apical surface called asymmetric unit membrane (AUM), comprised of uroplakin proteins that provide a barrier against re-absorption of urine (thus the term "umbrella cells") [22].

The bladder urothelium has in the midst of the slowest turnover rates of any adult tissue [23,24]. By way of responding to injury, for example, as a consequence of bacterial infection or exposure to toxins, the urothelium undergoes rapid proliferation and ultimately regenerates an intact urothelium [25,26], although the actual response may depend upon the specific inducing agent [27]. The implication of these observations is that the adult urothelium contains stem or progenitor cells that are capable of its regeneration. Such stem or progenitor cells have long been thought to reside in the basal cell layer. Especially, lineage tracing of mouse bladder following pathogen-induced regeneration demonstrated that basal cells give rise to all urothelial cell types, supporting their progenitor role [28].

Most (~90%) are urothelial carcinomas, which are the subject of this review and are referred to simply as "bladder cancers" completely. The remainder (~10%) include primary squamous cell carcinoma, adenocarcinoma, small cell carcinoma, or sarcomatoid carcinoma, which are not discussed additional in this review [29–31].

The integration of RNA subtype classification, pathway information, epithelial to mesenchymal transition (EMT) and carcinoma in situ (CIS) signatures and immune infiltrate analyses leads us to propose a model of mRNA-based expression subtypes that may be associated with a unique response to therapies and can be prospectively tested in clinical trials (Table 1). Personalized therapies could help optimize the patient's overall outcome while preventing unnecessary toxicity for those who do not respond. The following observations generate hypotheses and are therefore not ready for use in clinical decision making [32].

There are several different molecular subtypes that help us understand and be able to develop different mouse models which recapitulate specific features or cancer subtypes.

The luminal-papillary subtype (35%) is characterized by:

- FGFR3 mutations;
- fusions with TACC3, and/or amplification;
- papillary histology;
- active sonic hedgehog signaling; and by low CIS scores;
- low risk for progression;
- preliminary data suggest a low likelihood of response to cisplatin-based NAC (neoadjuvant chemotherapy) (Seiler et al., 2017).

The luminal-infiltrated subtype (19%) is characterized by:

- the lowest purity, with a high expression of EMT and myofibroblast markers;
- medium expression of the immune markers CD274 (PD-L1) and CTLA4;
- has been reported to respond to immune checkpoint therapy with atezolizumab in patients with metastatic or unresectable bladder cancer (Rosenberg et al. 2016);

The luminal subtype (6%) is characterized by:

- high expression of luminal markers, as well as KRT20 and SNX31;

The basal-squamous subtype (35%) is characterized by:

- higher incidence in women;
- squamous differentiation;

- basal keratin expression;
- high expression of immune markers CD274 (PD-L1) and CTLA4 and other signs of immune infiltration.

  The neuronal subtype (5%) is characterized by:

- the expression of both neuroendocrine and neuronal genes;
- do not have the typical morphological characteristics associated with neuroendocrine tumors;
- Etoposide-cisplatin therapy is recommended in neoadjuvant and metastatic settings.

Robertson AG et al. identified 34 additional significantly mutated genes (SMGs) and 158 genes that are subject to epigenetic silencing. All of these may offer additional potential therapeutic targets, fusion events that implicate PPARG as a key gene in bladder cancer development, and refined subtypes defined by considering both miRNA and lncRNA profiling MIBC. APOBEC expression and activity in the normal bladder could lead to preventive strategies that target APOBEC as a key mutagenic source in bladder cancer. Therapeutic opportunities target chromatin modifier gene mutations which are common in bladder cancer through rebalancing acetylation and deacetylation, and through other chromatin modifications [32].

Table 1. Proposed schematic illustration of the expression-based, subtype-stratified therapeutic approach as a framework for prospective hypothesis testing in clinical trials. Adapted from A. Gordon Robertson, Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer, 2017, Cell 171 [32]).

Classification	Subtype	Characteristics	Therapy
Luminal KRT20+ GATA3+ FOXA1+	Luminal-papillary	FGFR3 mut, fusion, amp Papillary histology SHH+ Low CIS	Low risk NAC (low predicted likelihood of response) FGFR3 inhibitors
	Luminal-infiltrated	Low purity EMT markers (TWIST1, ZEB1) miR-200 family Medium CD274 (PD-L1), CTLA-4 Myofibroblast markers Wild type p53	Anti-PD-L1, PD-1, CTLA-4 Cisplatin-based NAC (low response rate)
	Luminal	UPKs KRT20 SNX31	Targeted therapy?
Basal/Squamous KRT5,6,14+ GATA3- FOXA1-	Basal/Squamous	Female Squamous differentiation Basal keratin markers High CD274 (PD-L1), CTLA4 Immune infiltrates	Anti-PD-L1 PD-1 CTLA-4 Cisplatin-based NAC
Neuronal	Neuronal	SOX2 DLX6 MSI1 PLEKHG4B E2F3/SOX4 amp High cell cycle	Etoposide/Cisplatin NAC

KRT20—Keratin 20; GATA3—(guanine adenine thymine adenine) binding protein 3; FOXA1—Forkhead box protein A1; KRT5,6,14—keratins 5,6,14; FGFR3—fibroblast growth factor receptor 3; SHH - Sonic Hedgehog; CIS—carcinoma in situ; EMT markers—Epithelial-Mesenchymal Transition markers; TWIST1—Twist-related protein 1; ZEB1—zinc-finger-enhancer protein 1; miR-200 family—microRNAs; CD274 (PD-L1)—CD274 gene (Programmed death-ligand 1); CTLA-4—Cytotoxic T-lymphocyte-associated Antigen 4; UPKs—uroplakins; SNX31—sorting nexin 31; SOX2—Sex Determining Region Y-box 2; DLX6—distal-less homeobox 6; MSI1—Musashi1; PLEKHG4B—pleckstrin homology and RhoGEF domain containing G4B; E2F3/SOX4— E2F transcription factor 3/Sex Determining Region Y-box 2; NAC—neoadjuvant chemotherapy; Anti-PD-L1—anti Programmed death-ligand 1; PD-1—Programmed cell death protein 1.

2. Bladder Cancer In Vivo Models

An ideal animal model of bladder cancer should recapitulate its human counterpart with the natural course of tumor growth, progression, and similar histopathological features. Most importantly, these models should possess a high reproducibility, predictive and translatability value to allow mechanistic, chemo-preventive, and therapeutic studies that can be furthered into human clinical trials. The most commonly used species for animal research are small rodents, such as mice and rats. Rodents have a lower urinary tract, similar to that of humans. Bladder cancer is not very common in rodents unless they are induced by a chemical carcinogen [33] or oncogenes [34]. Mouse strains such as C57B6, BALBC, and ICR, and rat strains such as Wistar, Sprague-Dawley, and Fisher are most commonly used for bladder cancer research [35]. Rat strains such as Brown Norway and DA/Han show a high incidence of spontaneous bladder tumors and can therefore be used as experimental models without treatment with chemical carcinogens [36]. Larger animals such as dogs, rabbits, guinea pigs, and hamsters have all been used in the past as bladder cancer models [37], but their use is limited due to financial and ethical constraints.

Animal models of bladder cancer can be categorized as autochthonous (spontaneous) and non-autochthonous (transplantable). The first are either chemically induced models or genetically engineered models. The transplantable models can be further sub-classified as syngeneic (murine bladder cancer cells implanted into immunocompetent or transgenic mice) and xenografts (human bladder cancer cells implanted into immune-deficient mice). These models can be further divided based on the site of the tumor as orthotopic (tumor growth occurs within the bladder) and heterotopic (tumor growth occurs outside of the bladder).

3. Autochthonous (Spontaneous) Models

3.1. Carcinogen Induced Model

Bladder cancer is caused by continuous exposure to chemical carcinogens such as tobacco, aromatic amines, and chlorinated hydrocarbons [38]. It is very important to examine the relationship between chemical carcinogenesis and the development of urothelial carcinoma of the bladder. The first urothelial carcinogenesis model was induced in rats [39–41]. Urothelial carcinoma can be caused predominantly in rodents with the use of several chemical carcinogens. The major chemical urothelial carcinogens are N-Butyl-N-(4-hydroxybutyl) nitrosamine (BBN), N-[4-(5-nitro-2-furyl)-2- thiazolyl] formamide (FANFT), and N-methyl-N-nitrosourea (MNU). Most of these carcinogenic agents have aromatic amine components.

BBN was first identified as a bladder carcinogen in rodents [40,42] and is detected in infectious metabolites, tobacco smoke, and the environment [35]. BBN-induced cancer models can be established either orally by adding it to drinking water and are degraded to N-butyl-N-(3-carboxypropyl)-nitrosamine, which has carcinogenic effects on the urothelium when cleared in the urine [35]. BBN-exposed mice develop various pathological features, including hyperplasia, dysplasia, CIS, and invasive tumors, as well as metastases that are histologically and genetically similar to human bladder tumors arising from extensive tobacco use [35]. Williams et al. [43] made a comparison of gene expression profiles of urothelial carcinoma for three different species: mouse, rat, and human. Several human genes homologous to those differentially expressed in carcinogen-induced rodent tumors were also differentially expressed in human bladder cancer and were associated with progression to muscle-invasive disease. BBN tumors showed overexpression of markers of basal cancer subtype and had a high mutation burden with frequent Trp53 (80%), Kmt2d (70%), and Kmt2c (90%) mutations by exome sequencing, similar to human muscle invasive bladder cancer (MIBC) [44]. The overall gene expression profiles of rodent tumors were tracked with those of invasive human tumors rather than those of non-muscle-invasive tumors. The cell cycle-associated genes such as cell division cycle 20 (CDC20), cell division cycle 2 (CDC2), cyclins D1 and B2 (CCND1 and CCNB2), mitotic arrest–deficient 2, Saccharomyces cerevisiae, homolog-like 1 (MAD2L1), and cyclin A2 (CCNA2) were primarily

differentially expressed between normal and cancerous urothelium in all three species [40]. BBN-induced rodent tumors, predominantly mouse tumors, have p53 mutations, or mutations in genes related to the p53 pathway, especially in high-grade tumors [45,46]. BBN chemical carcinogenesis model in transgenic mice demonstrated to be a powerful tool to identify the mechanism of action of tumor suppressor and oncogenes as well as the pre-neoplastic lesions that are not clinically encountered [47]. The role of the matricellular glycoprotein, secreted protein acidic and rich in cysteine (SPARC) in the pathobiology of bladder cancer was reported [47]. BBN model can be also used to study the impact of conditional cell/tissue-specific knockout/knock-in of genes. This is the result of the conditional knockout of estrogen receptor α and β [48], as well as androgen receptor [49]. Additionally, this model has been used to evaluate the preventive and therapeutic efficacy of chemotherapeutics [50–53].

MNU is another genotoxic carcinogen that acts directly on the urothelium by intravesical instillation and leads to constitutive DNA methylation [54]. The primary advantage of this model is that papilloma and carcinoma appear after 12 weeks and MNU is the only carcinogen to produce bladder cancer at a single dose [41]. MNU is intrinsically unstable and should be stored at a low temperature and protected from light. Moreover, it may result in experimental inconsistencies due to its decomposition and altered carcinogenic potency over time.

FANFT is an indirect chemical carcinogen that stimulates the bladder mucosa, and bladder tumors mostly develop into transitional cell carcinoma (TCC) after being fed to rodents for 5 to 8 months [55]. FANFT is not currently common as it is identified as an environmental pollutant and is hazardous for human health.

3.2. Genetically Engineered Models

The transgenic mouse or genetically engineered mouse (GEM) represent useful research systems that are engineered to carry cloned oncogenes or lack tumor-suppressing genes and allow the investigation of human disease-associated genetic abnormalities in vivo. Several methods are employed to generate GEM models.

Mice with germline deletion may not allow investigation of the gene function if knockout leads to premature death or embryonic lethality and do not allow a clear distinction of the tissue/cell-specific contribution of a given gene in the disease. An approach that avoids these limitations is a conditional gene knock-in or knockout involving the Cre-loxP system, which allows the study of genetic function in specific cell and tissue types [56]. Urothelial-specific Cre system is available and is typically used to selectively achieve gene knockin/knockout in the bladder epithelium. The Cre-loxP system is based on the bacteriophage P1 wherein Cre recombinase acts on palindromic sequences called loxP sites that have been genetically engineered into the specific sites in the mouse genome. Cre recombinase can then excise the genomic sequence between two loxP sites. As a consequence, mouse alleles containing loxP sites can be used for introducing mutations, or for a temporally or spatially controlled gene knockout. The majority of the bladder cancer GEM models have used the mouse Uroplakin II (UpkII) promoter which is the 3.6 kb 5′-upstream sequence of mouse UpkII gene [57]. Zhang et al. demonstrated that SV40 (Simian virus 40) mice generated using UPII promoter developed CIS urothelial carcinoma with a low copy number of SV40T transgene and those with high copies developed CIS along with invasive and metastatic transitional carcinoma [58]. GEM models are widely used for investigating specific gene functions including, H-RAS, p53, RB, PTEN, fibroblast growth factor receptor (FGFR), and epidermal growth factor receptor (EGFR), in the development of bladder cancer.

Another approach to conditionally manipulate genes in the urothelium is the direct injection of adenovirus- Cre in the lumen of the bladder. This method has been used to investigate the roles of the main driver mutations such as K-ras, p53, Pten, and Rb in the temporal and spatial development of urothelial cancer [59–61]. These studies further identified therapeutic targets downstream of the aforementioned mutations. Yang and

colleagues [62] identified that the simultaneous inactivation of p53 and activation of K-ras induced the quick formation of spindle-cell sarcoma in the soft tissues adjacent to the bladder but the slow formation of urothelial hyperplasia inside the bladder. These results strongly show that the effect of oncogene regulation to produce either hyperplasia or carcinogenesis greatly depends on the type of tissue. Most importantly, studies with GEM models that recapitulate disease progression allowed the preclinical validation of the key pathways involved in urothelial cancer progression from non muscle invasive (NMI) to muscle invasive (MI) disease [59,60,62,63] and paved the way to clinical trials for intra-vesical delivery of single or combinatorial chemotherapeutics to prevent disease progression in high-risk patients with early-stage disease [64].

3.3. Non-Autochthonous (Transplantable) Models

The transplantable models (xenografts and syngeneic) can be categorized as heterotopic and orthotopic models based on the site of tumor cell implantation. It is essential to choose representative and reproducible cell lines for transplantable models. A large number of human and rodent urinary bladder cancer cell lines are available representing different origins, grades, and stages of urothelial carcinoma, and mirror many of the genetic, morphologic, and gene expression alterations observed in human urothelial carcinoma [65]. Several cell lines were established from invasive and metastatic tumors, which are advantageous in the investigation of late tumor progression and metastatic lesions [66,67].

It is recommended to purchase cell lines from authenticated cell repositories. Identity verification with short tandem repeat (STR) profiling establishes a DNA fingerprint for every human cell line and may be used as a record of the line. STR profiling uses multiplex polymerase chain reaction (PCR) to simultaneously amplify several polymorphic markers in the human genome. Each cell line exhibits a pattern of repeats which constitutes the unique STR identity profile [68].

3.4. Orthotopic Models

Orthotopic models allow the evaluation of tumor behavior in an organ-specific microenvironment. These models can be realized by injecting urothelial cancer cells into the lumen of the bladder of recipient hosts. Transitional cells are preferentially established on an altered urothelial surface. For this purpose, the natural protective epithelium lining the surface of the urinary bladder has to be damaged to facilitate tumor cell adhesion. The mechanical damage can be achieved by electrical cauterization and epithelial abrasion [69–75], as well as chemical denudation with HCl [76,77], N-methyl-N-nitrosourea [78], silver nitrate [69] followed by tumor cell instillation.

Several factors determine the efficient tumor take rate, such as the bladder preconditioning, cell concentration, the instillation volume, and the tumor cell dwell time in the bladder [79]. An instillation volume of 50–100 µL is typically used with mice weighing <30 g. Higher volumes can result in peri-urethral leakage and reflux to the upper urinary tract. Therefore, it is highly suggested to ensure complete emptying of the bladder before instillation as it prevents over-distension of the mouse bladder [79]. It has been previously reported that an increase in the tumor cell dwell time augments the duration for which the cancer cells are in contact with the bladder mucosa, resulting in an increase in the tumor inoculation rate [80,81]. Apart from technical limitations, orthotopic models metastasize only to local lymph nodes and do not allow studies of metastasis or recurrence.

3.5. Heterotopic Transplantable Models

These models represent the injecting/transplanting rodent or human bladder cancer cells or tissues in a rodent in an ectopic site other than a tissue of origin. These may be syngeneic (rodent cells/tissue in immunocompetent or transgenic rodent) or xenografts (human cells/tissue in immuno-deficient mice). These models are easy to manage, easy to establish, cost-effective, and are widely used in mechanistic studies as well as in evaluating

the efficacy of novel therapeutic agents [82]. Several transplantable models are used in complementary ways to study different aspects of tumor growth and metastasis, as well as the effect of tumor-stromal interactions [62].

3.6. Syngeneic Models

Syngeneic models are created by implanting rodent bladder cancer cells or tissues into syngeneic, immunocompetent, or transgenic animals. Rodent bladder cancer cell lines such as AY27 and MBT2 were initially induced by administering C3H/He mouse and Fischer 344 rat strains with FANFT) [76,83], whereas MB49 was induced by feeding 7,12-Dimethylbenzanthracene to the C57BL/6 mouse strain [84]. Implantation of urothelial cancer cells in syngeneic hosts may be orthotopic (intravesical) or heterotopic (subcutaneous, renal capsule implantation, or experimental metastasis model). For example, the use of the immunocompetent host in syngeneic models allows the study of intravesical BCG treatment or gene therapy [85–88]. Loskog et al. generated a subcutaneous mouse model using MB49 cells in C57BL/6 female and male mice [89]. Another spontaneous metastatic model using syngeneic MB49 cell line revealed the significance of the vasoconstrictor protein endothelin-1 (ET-1) in the early establishment of metastases. This study revealed that circulating tumor cells generate a strong inflammatory response in the lungs mediated by the ET-1/endothelin-1 receptor A (ETAR) axis and this allows metastatic colonization. Moreover, pharmacological inhibition of ETAR by ZD4054 prior to injection of tumor cells significantly decreased the early inflammatory response as well as the development of lung metastases. Therefore, this spontaneous metastatic model allowed us to determine that tumor ET-1 expression and ETAR activity are essential for metastatic lung colonization, but their functional role is less significant in established primary or metastatic tumors. These data provide major evidence that preclinical evaluation of new therapies should be conducted in adjuvant settings using metastatic colonization models [90]. The significance of tumor expression of the proteoglycan versican and chemokine CCL2 (also known as MCP1) in promoting lung metastasis of bladder cancer was investigated using the syngeneic MB49 metastatic model [91]. The murine MB49 bladder tumor model is quite similar to human bladder cancer, making it an interesting model for studying novel genes and immunotherapies.

Syngeneic models are both time and cost-effective, and reproducible. These model systems proved to be very useful in developing and validating new therapies at targeting the multistep cascade of tumor growth, progression, spontaneous metastasis, and organotropism.

3.7. Xenograft Models

Xenografts depict the implantation of human tumor cells or tissues in immunocompromised mice. Xenografts can be subdivided into: orthotopic (intravesical) or heterotopic (subcutaneous, renal capsule implantation, or intravenous or intracardiac for experimental metastasis).

3.8. Orthotopic Xenografts

Chade et al. [68] described the use of intravesical 0.2% trypsin before treatment for 30 min and mechanical bladder injury immediately before cancer cells are instilled for 3 h. Microscopic examination of the bladder 10 days after implantation revealed bladder tumors in 80–100% of mice, but the procedure-related death was high. Jager et al. [92] developed a novel high precision approach for orthotopic xenograft implantation. Bladder cancer cell lines such as UMUC1, UMUC3, and UMUC13 were inoculated into 10-week-old athymic nude mice by percutaneous injection under ultrasound guidance. This model enabled the monitoring of tumor volume, the measurement of in vivo tumor perfusion with microbubble contrast agents, and the injection of therapeutic agents into the tumor under ultrasound guidance. Orthotopic tumors exhibit an increased microvessel density, high growth factors expression, and proteolytic enzyme activity compared to those of

subcutaneous tumors. Moreover, rodents have a lower urinary tract which is comparable to humans, and neoplasms of the bladder are morphologically very similar, with a phenotype similar to human urinary carcinoma with respect to tumorigenesis and gene expression.

3.9. Subcutaneous Tumor Xenografts

Human cancer cells are implanted in the flank or hind leg of immune-deficient mice. The subcutaneous model is the most commonly adopted model. This model is easy to establish, easy to manage, cost-effective, and is used in mechanistic studies, and in evaluating the efficacy of novel therapeutic agents [93]. Another advantage of subcutaneous tumors is that they can be used to study local recurrence after excision. However, since subcutaneous tumors are not established at the original site, the urinary bladder, they do not recapitulate the real tumor microenvironment. Subcutaneous xenografts do not metastasize, therefore, they cannot be used to study certain aspects of tumor biology specifically metastasis [94–96].

3.10. Experimental Metastasis Models

These models are used to study the mechanism of metastasis after tail vein or intracardiac injection of human cancer cells directly into the circulation. The inoculated cells require an initial adaptation phase in which the tumor cells acclimate to a new microenvironment. Cells that survive the turbulence in circulation grow and metastasize in distant organs, such as the lungs [97,98]. The developed metastases can be isolated and cultured in vitro as metastatic derived isogenic cell clones that exhibit higher tumorigenic and metastatic potential compared to the parental cell line [97,98].

3.11. Hollow Fiber (HF) Model

This model utilizes semipermeable biocompatible fibers that can be loaded with cancer cells and implanted surgically in animals, which can be treated by chemotherapeutic agents [99–101]. Moon et al. [102] filled polyvinylidene fluoride (PVDF) hollow fibers with human bladder cancer cell lines (CRL2742, 253JP, SW1710, HTB9) and surgically implanted these fibers subcutaneously and intraperitoneally into athymic nude mice. These mice were treated with gemcitabine, cisplatin, paclitaxel and after 6 days these fibers were recovered to determine cell viability. Although tedious, it is a cost-effective screening method because multiple cell lines can be evaluated for drug cytotoxicity within a short duration (assay time < 2 weeks).

4. Modeling Bladder Cancer in Mice

Although recent studies have advanced our conceptual understanding of the biological, molecular, and environmental factors associated with bladder cancer, this knowledge has not yet advanced to the point of impacting patient care.

Our understanding of these issues would greatly benefit from the availability of mouse models that accurately represent specific phenotypes or subtypes of bladder cancer and are based on relevant genes/pathways/processes that are associated with bladder cancer.

An important distinction between the types of models is that carcinogen-based and GEM models are autochthonous, which means that tumors originate in the bladder, whereas graft models are non-autochthonous since they are implanted into recipient hosts. Notably, graft recipient mice are usually immunodeficient, which is of relevance given the known importance of the immune system for cancer progression and metastasis [103].

However, engraftment models have the considerable advantage of their relative ease and rapidity of generation and use for analyses of the functional relevance of candidate genes. Furthermore, although they are both autochthonous, tumors in carcinogen-based models are, by definition, induced by carcinogens, whereas those in GEM models arise following the manipulation of specific genes. Thus, these different approaches for modeling bladder cancer in mice are highly complementary.

5. Non-Muscle Invasive Versus Muscle Invasive Bladder Cancer

Several lines of evidence support the general concept that the distinct clinical outcomes of low-grade non-muscle-invasive versus high-grade muscle-invasive bladder tumors reflect their distinct molecular causes and, as discussed above, potentially distinct cells of origin. Indeed, certain molecular alterations, such as gain of function mutations of FGFR3, are prevalent in low-grade non-muscle invasive bladder cancers whereas other alterations, such as p53 loss or mutation, are prevalent in high-grade muscle-invasive bladder cancers [104–107].

Analyses of gene expression profiling [108–113] and/or genomic alterations [111,114–118] have supported the general concept that low-grade non-invasive versus high-grade invasive bladder tumors are molecularly distinct and it is difficult to fully reconcile a mutual-exclusivity model considering that some superficial bladder tumors can progress to invasive disease. Meta-analysis of expression profiling data from non-invasive and invasive bladder cancers failed to identify molecular subtypes that are clearly associated with the pathological stage [119]. Furthermore, recent whole genome sequencing and transcriptome analyses comparing low-grade and high-grade bladder cancers support the concept that these evolve in parallel, rather than mutually exclusive [120].

Thus, low-grade non-muscle invasive and high-grade muscle-invasive bladder cancer may be viewed more appropriately as broadly distinct entities along a continuum of disease progression. In this framework, the actual phenotype and outcome may reflect the culmination of molecular alterations that tend to drive more or less aggressive phenotypes, distinct cells of origin, which may contribute to tumor aggressiveness, and potential interactions with environmental exposures, such as smoking, carcinogens, or inflammation.

6. Patient Derived Xenografts

Engraftment of patient-derived tumor tissues into immunodeficient mouse hosts (called patient-derived xenograft—PDX—models) [121], which have been described for bladder cancer [122].

Since PDX models are derived from individual patient tumors, the expectation is that the resulting tumors capture the unique genomic and molecular properties of the individual patient from which they are derived; the further expectation is that PDX models should enable analyses of clinical responses based on the unique characteristics of a given tumor. Indeed, preclinical studies using PDX models of bladder cancer have supported the concept that co-targeting PI3K and MAP signaling may be beneficial for certain types of bladder cancers [123].

A few reports have described the generation of PDX models for bladder cancer; thus, it is not clear whether such models can be generated efficiently or whether they will indeed capture all or most bladder cancer subtypes. On the other hand, whereas the generation of PDX models for certain types of cancers (such as prostate cancer, for example) may be limited by tissue availability, this should primarily not be a consideration for bladder cancer because primary tissue is readily available from TUR as well as cystectomy. Thus, if indeed bladder cancer has a reasonable 'take-rate' in the recipient hosts, it should be feasible to generate a range of PDX models, ideally representative of the various subtypes of bladder cancer.

In terms of genetic fidelity, parental tumor and PDX generally have the most (>90%) genetic alterations in urothelial carcinoma [124]. The histological features of the original tumor are preserved for three to six passages [124,125]. Potential therapeutic targets in PDX bladder cancer have been identified using fluorescence-activated cell sorting (FACS) techniques. The combined use of FACS and PDX models has led to successful molecular profiling and the identification of a gene signature of bladder tumor-initiating cells that has been associated with a poor prognosis [126]. These studies also highlighted the role of cancer stem cells in therapeutic resistance in bladder cancer using PDX, finding that CK14+ cells contribute to tumor regrowth by activating a proliferation response

after chemotherapy-induced damage, and these cells demonstrated the functional criteria necessary to be considered cancer stem cells [16,127].

PDX models are widely used as screening platforms for clinical drug trials. Advances in experimental methods have shown that tumor heterogeneity is a hallmark of cancer, as is bladder cancer [122]. Because PDX avoids the use of an in vitro process, PDX models are expected to recapitulate the complexity of human cancer, with preserved tumor heterogeneity, cellular lineage hierarchy, and tumor–stroma interaction. Indeed, a high correlation of drug response has been reported between PDX models and patients [124,128].

One of the drawbacks of PDX models is the inevitable requirement of immunodeficient mice to prevent immune attacks against xenograft tumors. The use of immunodeficient mice as PDX hosts has several limitations. First, complex microenvironments of human primary tumors are not recapitulated in immunodeficient mice. Despite the successful growth of primary human tumors in immunodeficient mice, the microenvironment, including tumor-promoting cells, is replaced by a murine equivalent. In this case, drugs targeting human cells may not work properly. Validation studies of PDX models have found a significant stromal loss of tumor after multiple murine transplantations [129,130]. Another limitation of immunodeficient mice is that graft-versus-host and anticancer immunity may not be completely interrupted during the PDX process, leading to engraftment failure [131]. In addition, lymphangiogenesis and angiogenesis precipitated by B lymphocytes and macrophages may influence tumor growth and metastasis [132,133]. Once a requirement for tumor microenvironment interaction has disappeared, leading to the unavailability of evaluating anticancer drugs that have an effect on the immune system or immunotherapies [129].

7. Monitoring and Evaluation of Animal Models for Bladder Cancer

Methods for tumor growth evaluation and therapeutic effects in animal models depend on the used model system. In subcutaneous tumors, measurement of the subcutaneous tumor mass and imaging of tumor cells labeled with a luminescent or a fluorescent tag are instrumental for monitoring tumor growth and evaluating the diagnosis and responses summarized. Detection of orthotopic and spontaneous urethral tumors cannot rely only on palpable bladder mass, weight loss, and urinalysis. A transurethral mini cystoscope is used as a non-invasive method for detecting and monitoring superficial tumors [134]. White light cystoscopy used in the clinic is unreliable for differentiation between low- vs high-grade cancer. It can neither assess the level of invasion or CIS nor differentiate CIS from inflammation [135]. The combination of mini trans-urethral cystoscopy with optical imaging modalities and intravesical injection of fluorescent or photosensitive dyes as fluorescein or proto-porphyrin precursors significantly improved the diagnostic value of mini-cystoscopy [104]. Other conventional imaging methods in animal studies include abdominal ultrasound, positron emission tomography-computerized tomography (PET-CT), and magnetic resonance imaging (MRI) and can be used for early tumor detection as early as 14 days after tumor inoculation in mice [136]. High-frequency and high-resolution intravesical ultrasound (HRUS) has been reported to demonstrate high sensitivity in monitoring tumor growth in orthotopic bladder cancer mouse models as a rapid, efficient, and comparatively inexpensive imaging modality [137–139]. Photoacoustic imaging (PAI) is a hybrid imaging modality that couples optical and ultrasound imaging in real time [140]. PAI can provide a better resolution than optical imaging for depths greater than 1 mm. Bioluminescence (BLI) is another commonly used optical imaging modality for both in vitro and in vivo non-invasive monitoring of molecular and cellular activities. BLI is considered to be a far superior imaging methodology compared to fluorescence, as BLI does not need excitation and avoids the auto-fluorescence background signal, and can be combined with other modalities as a theragnostic approach [12,139,140].

8. Conclusions

Mouse models of urothelial cancer of the bladder (Figure 2) present useful tools for advancing early diagnosis of the disease, implementing tests for high-risk populations for primary prevention and early diagnosis, and stratification of patients for a given therapy. They can be exploited not only to identify early diagnostic biomarkers but also to identify biomarkers related to specific genetic factors associated with the human disease. The use of transgenic and genetically engineered mouse models can help recognize the challenges associated with modeling tumor heterogeneity, tumor–stromal interactions, and the contribution of the stromal compartment and the immune system to disease progression. The use of transplantable models (syngeneic and xenografts) can be combined to carefully study the events in multistep cascades of cancer progression, invasiveness, and metastasis.
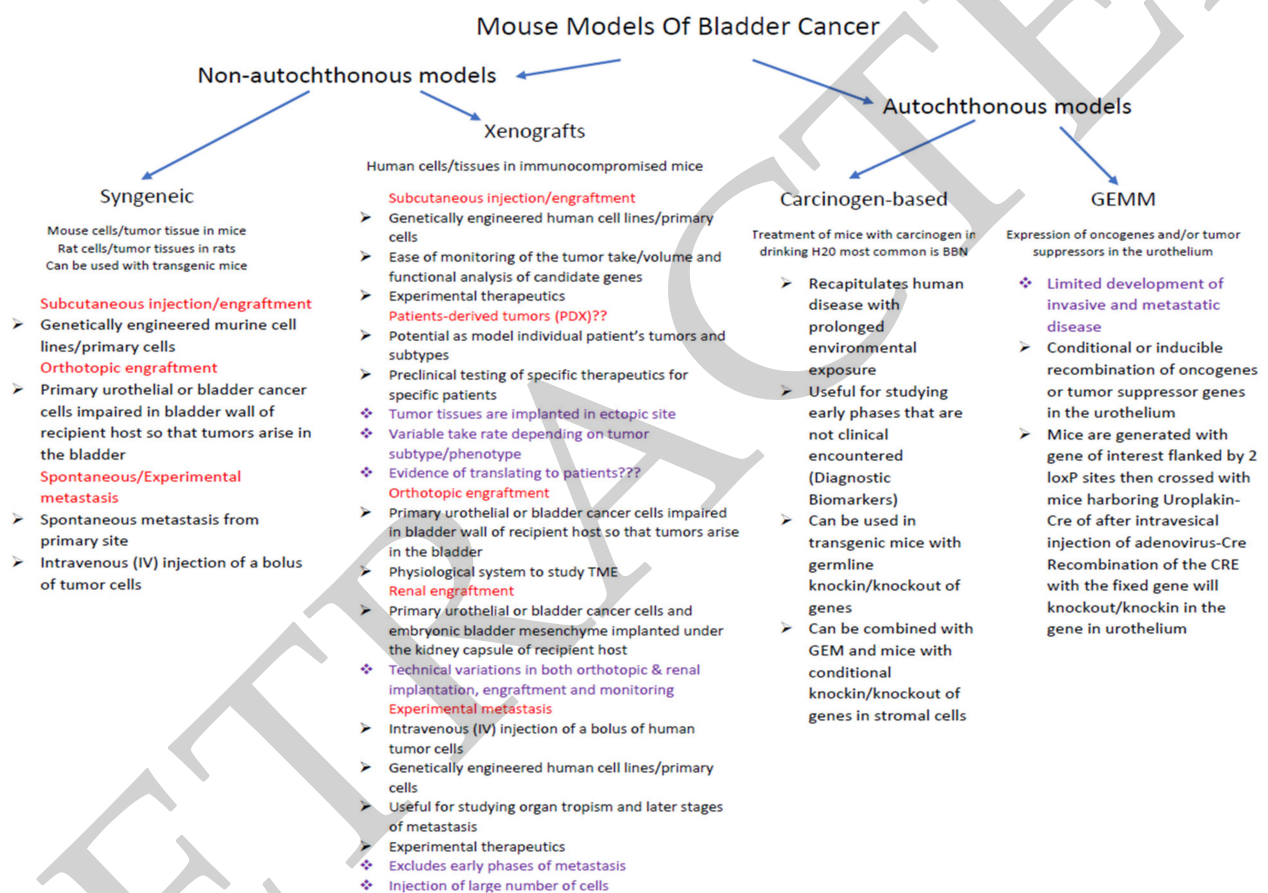

Figure 2. Summary of the available mouse models of urinary bladder cancer (© Bincy Anu John1 and Neveen Said, Insights from animal models of bladder cancer: recent advances, challenges, and opportunities, Oncotarget, 2017, Vol. 8, (No. 34) [12]).

Combining multiple models with imaging technologies will enable the generation of robust models that can further our knowledge of the pathobiology of the disease and allow the discovery and validation of novel diagnostic and prognostic biomarkers and personalized therapies.

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References

1. Siegel, R.; Naishadham, D.; Jemal, A. Cancer statistics, 2013. *CA Cancer J Clin.* **2013**, *63*, 11–30. [CrossRef]
2. De George, K.C.; Holt, H.R.; Hodges, S.C. Bladder cancer: Diagnosis and Treatment. *Am. Fam. Physician* **2017**, *96*, 507–514.
3. Letašiová, S.; Medveďová, A.; Šovčíková, A.; Dušinská, M.; Volkovová, K.; Mosoiu, C.; Bartonová, A. Bladder cancer, a review of the environmental risk factors. *Environ. Heal.* **2012**, *11*, S11. [CrossRef]
4. Andrew, A.S.; Schned, A.R.; Heaney, J.A.; Karagas, M.R. Bladder cancer risk and personal hair dye use. *Int. J. Cancer* **2004**, *109*, 581–586. [CrossRef]
5. Jankovic, S.; Radosavljevic, V. Risk factors for bladder cancer. *Tumori J.* **2007**, *93*, 4–12. [CrossRef]
6. Aben, K.K.; Baglietto, L.; Baffoe-Bonnie, A.; Coebergh, J.-W.W.; Bailey-Wilson, J.E.; Trink, B.; Verbeek, A.L.; Schoenberg, M.P.; Witjes, J.A.; Kiemeney, L.A. Segregation analysis of urothelial cell carcinoma. *Eur. J. Cancer* **2006**, *42*, 1428–1433. [CrossRef] [PubMed]
7. Kantor, A.F.; Hartge, P.; Hoover, R.N.; Fraumeni, J.F., Jr. Familial and environmental interactions in bladder cancer risk. *Int. J. Cancer* **1985**, *35*, 703–706. [CrossRef] [PubMed]
8. Murta-Nascimento, C.; Silverman, D.T.; Kogevinas, M.; Garcia-Closas, M.; Rothman, N.; Tardon, A.; Garcia-Closas, R.; Serra, C.; Carrato, A.; Villanueva, C.; et al. Risk of bladder cancer associated with family history of cancer: Do low-penetrance polymorphisms account for the increase in risk? *Cancer Epidemiol. Biomark. Prev.* **2007**, *16*, 1595–1600. [CrossRef] [PubMed]
9. Bellamri, M.; Brandt, K.; Brown, C.V.; Wu, M.-T.; Turesky, R.J. Cytotoxicity and genotoxicity of the carcinogen aristolochic acid I (AA-I) in human bladder RT4 cells. *Arch. Toxicol.* **2021**, *95*, 2189–2199. [CrossRef] [PubMed]
10. Dinney, C.P.; McConkey, D.J.; Millikan, R.E.; Wu, X.; Bar-Eli, M.; Adam, L.; Kamat, A.M.; Siefker-Radtke, A.; Tuziak, T.; Sabichi, A.L.; et al. Focus on bladder cancer. *Cancer Cell* **2004**, *6*, 111–116. [CrossRef] [PubMed]
11. Rebhan, K.; Ertl, I.E.; Shariat, S.F.; Grollman, A.P.; Rosenquist, T. Aristolochic acid and its effect on different cancers in uro-oncology. *Curr. Opin. Urol.* **2020**, *30*, 689–695. [CrossRef] [PubMed]
12. John, B.A.; Said, N. Insights from animal models of bladder cancer: Recent advances, challenges, and opportunities. *Oncotarget* **2017**, *8*, 57766–57781. [CrossRef]
13. Catsburg, C.E.; Gago-Dominguez, M.; Yuan, J.-M.; Castelao, J.E.; Cortessis, V.K.; Pike, M.C.; Stern, M.C. Dietary sources of N-nitroso compounds and bladder cancer risk: Findings from the Los Angeles bladder cancer study. *Int. J. Cancer* **2014**, *134*, 125–135. [CrossRef] [PubMed]
14. Wu, X.-R. Urothelial tumorigenesis: A tale of divergent pathways. *Nat. Rev. Cancer* **2005**, *5*, 713–725. [CrossRef]
15. Hicks, R.M. The mammalian urinary bladder: An accommodating organ. *Biol. Rev.* **1975**, *50*, 215–246. [CrossRef]
16. Bradley, W.E.; Long, D.M. Morphology of the developing mammalian bladder. *Investig. Urol.* **1969**, *7*, 66–73.
17. Baskin, L.; Hayward, S.; Young, P.; Cunha, G. Ontogeny of the Rat Bladder: Smooth Muscle and Epithelial Differentiation. *Cells Tissues Organs* **1996**, *155*, 163–171. [CrossRef]
18. De La Rosette, J.; Smedts, F.; Schoots, C.; Hoek, H.; Laguna, P. Changing patterns of keratin expression could be associated with functional maturation of the developing human bladder. *J. Urol.* **2002**, *168*, 709–717. [CrossRef]
19. Khandelwal, P.; Abraham, S.N.; Apodaca, G. Cell biology and physiology of the uroepithelium. *Am. J. Physiol. Physiol.* **2009**, *297*, F1477–F1501. [CrossRef] [PubMed]
20. Castillo-Martin, M.; Domingo-Domenech, J.; Karni-Schmidt, O.; Matos, T.; Cordon-Cardo, C. Molecular pathways of urothelial development and bladder tumorigenesis. *Urol. Oncol. Semin. Orig. Investig.* **2010**, *28*, 401–408. [CrossRef]
21. Sun, W.; Aalders, T.W.; Oosterwijk, E. Identification of potential bladder progenitor cells in the trigone. *Dev. Biol.* **2014**, *393*, 84–92. [CrossRef] [PubMed]
22. Wu, X.-R.; Kong, X.-P.; Pellicer, A.; Kreibich, G.; Sun, T.-T. Uroplakins in urothelial biology, function, and disease. *Kidney Int.* **2009**, *75*, 1153–1165. [CrossRef]
23. Messier, B.; Leblond, C.P. Cell proliferation and migration as revealed by radioautography after injection of thymidine-H3 into male rats and mice. *Am. J. Anat.* **1960**, *106*, 247–285. [CrossRef]
24. Jost, S.P.; Potten, C.S. Urothelial Proliferation In Growing Mice. *Cell Prolif.* **1986**, *19*, 155–160. [CrossRef] [PubMed]
25. Cooper, E.H.; Cowen, D.M.; Knowles, J.C. The recovery of mouse bladder epithelium after injury by 4-ethylsulphonylnaphthalene-1-sulphonamide. *J. Pathol.* **1972**, *108*, 151–156. [CrossRef] [PubMed]
26. Lavelle, J.; Meyers, S.; Ramage, R.; Bastacky, S.; Doty, D.; Apodaca, G.; Zeidel, M.L. Bladder permeability barrier: Recovery from selective injury of surface epithelial cells. *Am. J. Physiol. Physiol.* **2002**, *283*, F242–F253. [CrossRef]
27. Mysorekar, I.U.; Isaacson-Schmid, M.; Walker, J.N.; Mills, J.C.; Hultgren, S.J. Bone Morphogenetic Protein 4 Signaling Regulates Epithelial Renewal in the Urinary Tract in Response to Uropathogenic Infection. *Cell Host Microbe* **2009**, *5*, 463–475. [CrossRef]
28. Shin, K.; Lee, J.; Guo, N.; Kim, J.; Lim, A.; Qu, L.; Mysorekar, I.U.; Beachy, P.A. Hedgehog/Wnt feedback supports regenerative proliferation of epithelial stem cells in bladder. *Nat. Cell Biol.* **2011**, *472*, 110–114. [CrossRef] [PubMed]

29. Kaufman, D.S.; Shipley, W.U.; Feldman, A.S. Bladder cancer. *Lancet* **2009**, *374*, 239–249. [CrossRef]

30. Prasad, S.M.; DeCastro, G.J.; Steinberg, G. Urothelial carcinoma of the bladder: Definition, treatment and future efforts. *Nat. Rev. Urol.* **2011**, *8*, 631–642. [CrossRef]

31. Dahm, P.; Gschwend, J.E. Malignant Non-Urothelial Neoplasms of the Urinary Bladder: A Review. *Eur. Urol.* **2003**, *44*, 672–681. [CrossRef]

32. Robertson, A.G.; Kim, J.; Al-Ahmadie, H.; Bellmunt, J.; Guo, G.; Cherniack, A.D.; Hinoue, T.; Laird, P.W.; Hoadley, K.; Akbani, R.; et al. Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. *Cell* **2017**, *171*, 540–556.e25. [CrossRef]

33. Clayson, D.; Fishbein, L.; Cohen, S. Effects of stones and other physical factors on the induction of rodent bladder cancer. *Food Chem. Toxicol.* **1995**, *33*, 771–784. [CrossRef]

34. Oliveira, P.A.; Colaço, A.; De la Cruz, L.F.; Lopes, C. Experimental bladder carcinogenesis-rodent models. *Exp. Oncol.* **2006**, *28*, 2–11.

35. Nóbrega, C.; Colaço, A.; Lopes, C.; Oliveira, P. Review: BBN as an urothelial carcinogen. *In Vivo* **2012**, *26*, 727–739.

36. van Moorselaar, R.J.; Ichikawa, T.; Schaafsma, H.E.; Jap, P.H.; Isaacs, J.T.; van Stratum, P.; Ramaekers, F.C.; Debruyne, F.M.; Schalken, J.A. The rat bladder tumor model system RBT resembles phenotypically and cytogenetically human superficial transitional cell carcinoma. *Urol. Res.* **1993**, *21*, 413–421. [CrossRef]

37. Crallan, R.; Georgopoulos, N.; Southgate, J. Experimental models of human bladder carcinogenesis. *Carcinogenesis* **2005**, *27*, 374–381. [CrossRef] [PubMed]

38. Babjuk, M.; Burger, M.; Zigeuner, R.; Shariat, S.F.; van Rhijn, B.W.; Compérat, E.; Sylvester, R.J.; Kaasinen, E.; Böhle, A.; Redorta, J.P.; et al. EAU Guidelines on Non–Muscle-invasive Urothelial Carcinoma of the Bladder: Update 2013. *Eur. Urol.* **2013**, *64*, 639–653. [CrossRef]

39. Druckrey, H.; Preussmann, R.; Ivankovic, S.; Schmidt, C.H.; Mennel, H.D.; Stahl, K.W. Selective induction of bladder cancer in rats by dibutyl- and N-butyl-N-butanol(4)-nitrosamine. *Z Krebsforsch.* **1964**, *66*, 280–290. [CrossRef]

40. Fukushima, S.; Hirose, M.; Tsuda, H.; Shirai, T.; Hirao, K. Histological classification of urinary bladder cancers in rats induced by N-butyl-n-(4-hydroxybutyl)nitrosamine. *Gan* **1976**, *67*, 81–90.

41. Williams, P.D.; Lee, J.K.; Theodorescu, D. Molecular Credentialing of Rodent Bladder Carcinogenesis Models. *Neoplasia* **2008**, *10*, 838–846. [CrossRef] [PubMed]

42. Fantini, D.; Glaser, A.P.; Rimar, K.J.; Wang, Y.; Schipma, M.; Varghese, N.; Rademaker, A.; Behdad, A.; Yellapa, A.; Yu, Y. A carcinogen-induced mouse model recapitulates the molecular alterations of human muscle invasive bladder cancer. *Oncogene* **2018**, *37*, 1911–1925. [CrossRef]

43. Masui, T.; Dong, Y.; Yamamoto, S.; Takada, N.; Nakanishi, H.; Inada, K.-I.; Fukushima, S.; Tatematsu, M. p53 mutations in transitional cell carcinomas of the urinary bladder in rats treated with N-butyl-N-(4-hydroxybutyl)-nitrosamine. *Cancer Lett.* **1996**, *105*, 105–112. [CrossRef]

44. Hicks, R.; Wakefield, J.S.J. Rapid induction of bladder cancer in rats with N-methyl-N-nitrosourea I. *Histology. Chem. Interact.* **1972**, *5*, 139–152. [CrossRef]

45. Cohen, S.M. Comparative Pathology of Proliferative Lesions of the Urinary Bladder. *Toxicol. Pathol.* **2002**, *30*, 663–671. [CrossRef]

46. Said, N.; Frierson, H.F.; Sanchez-Carbayo, M.; Brekken, R.A.; Theodorescu, D. Loss of SPARC in bladder cancer enhances carcinogenesis and progression. *J. Clin. Investig.* **2013**, *123*, 751–766. [CrossRef]

47. Garnett, M.J.; Edelman, E.J.; Heidorn, S.J.; Greenman, C.D.; Dastur, A.; Lau, K.W.; Greninger, P.; Thompson, I.R.; Luo, X.; Soares, J.; et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. *Nature* **2012**, *483*, 570–575. [CrossRef]

48. Johnson, D.T.; Hooker, E.; Luong, R.; Yu, E.-J.; He, Y.; Gonzalgo, M.L.; Sun, Z. Conditional Expression of the Androgen Receptor Increases Susceptibility of Bladder Cancer in Mice. *PLoS ONE* **2016**, *11*, e0148851. [CrossRef]

49. Jiang, T.; Liu, T.; Li, L.; Yang, Z.; Bai, Y.; Liu, D.; Kong, C. Knockout of phospholipase Cepsilon attenuates N-butyl-N-(4-hydroxybutyl) nitrosamine-induced bladder tumorigenesis. *Mol. Med. Rep.* **2016**, *13*, 2039–2045. [CrossRef] [PubMed]

50. Matsuo, T.; Miyata, Y.; Asai, A.; Sagara, Y.; Furusato, B.; Fukuoka, J.; Sakai, H. Green Tea Polyphenol Induces Changes in Cancer-Related Factors in an Animal Model of Bladder Cancer. *PLoS ONE* **2017**, *12*, e0171091. [CrossRef]

51. Shang, Z.; Li, Y.; Zhang, M.; Tian, J.; Han, R.; Shyr, C.-R.; Messing, E.M.; Yeh, S.; Niu, Y.; Chang, C. Antiandrogen Therapy with Hydroxyflutamide or Androgen Receptor Degradation Enhancer ASC-J9 Enhances BCG Efficacy to Better Suppress Bladder Cancer Progression. *Mol. Cancer Ther.* **2015**, *14*, 2586–2594. [CrossRef] [PubMed]

52. Gao, Y.; Shi, Q.; Xu, S.; Du, C.; Liang, L.; Wu, K.; Wang, K.; Wang, X.; Chang, L.S.; He, D.; et al. Curcumin Promotes KLF5 Proteasome Degradation through Downregulating YAP/TAZ in Bladder Cancer Cells. *Int. J. Mol. Sci.* **2014**, *15*, 15173–15187. [CrossRef]

53. Reis, L.O.; Pereira, T.C.; Fávaro, W.J.; Cagnon, V.; Lopes-Cendes, I.; Ferreira, U. Experimental animal model and RNA interference: A promising association for bladder cancer research. *World J. Urol.* **2009**, *27*, 353–361. [CrossRef]

54. Spry, L.A.; Zenser, T.V.; Cohen, S.M.; Davis, B.B. Role of renal metabolism and excretion in 5-nitrofuran-induced uroepithelial cancer in the rat. *J. Clin. Investig.* **1985**, *76*, 1025–1031. [CrossRef]

55. Nagy, A. Cre recombinase: The universal reagent for genome tailoring. *Genesis* **2000**, *26*, 99–109. [CrossRef]

56. Lin, J.H.; Zhao, H.; Sun, T.-T. A tissue-specific promoter that can drive a foreign gene to express in the suprabasal urothelial cells of transgenic mice. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 679–683. [CrossRef] [PubMed]

57. Zhang, Z.T.; Pak, J.; Shapiro, E.; Sun, T.-T.; Wu, X.-R. Urothelium-specific expression of an oncogene in transgenic mice induced the formation of carcinoma in situ and invasive transitional cell carcinoma. *Cancer Res.* **1999**, *59*, 3512–3517.

58. Puzio-Kuter, A.M.; Castillo-Martin, M.; Kinkade, C.W.; Wang, X.; Shen, T.H.; Matos, T.; Shen, M.M.; Cordon-Cardo, C.; Abate-Shen, C. Inactivation of p53 and Pten promotes invasive bladder cancer. *Genes Dev.* **2009**, *23*, 675–680. [CrossRef]

59. Seager, C.M.; Puzio-Kuter, A.M.; Patel, T.; Jain, S.; Cordon-Cardo, C.; Mc Kiernan, J.; Abate-Shen, C. Intravesical Delivery of Rapamycin Suppresses Tumorigenesis in a Mouse Model of Progressive Bladder Cancer. *Cancer Prev. Res.* **2009**, *2*, 1008–1014. [CrossRef]

60. Yang, X.; La Rosa, F.G.; Genova, E.E.; Huber, K.; Schaack, J.; DeGregori, J.; Serkova, N.J.; Li, Y.; Su, L.-J.; Kessler, E.; et al. Simultaneous Activation of Kras and Inactivation of p53 Induces Soft Tissue Sarcoma and Bladder Urothelial Hyperplasia. *PLoS ONE* **2013**, *8*, e74809. [CrossRef]

61. Kobayashi, T.; Owczarek, T.B.; McKiernan, J.M.; Abate-Shen, C. Modelling bladder cancer in mice: Opportunities and challenges. *Nat. Rev. Cancer* **2015**, *15*, 42–54. [CrossRef] [PubMed]

62. Delto, J.C.; Kobayashi, T.; Benson, M.C.; McKiernan, J.; Abate-Shen, C. Preclinical analyses of intravesical chemotherapy for prevention of bladder cancer progression. *Oncotarget* **2013**, *4*, 269–276. [CrossRef]

63. Gabriel, U.; Bolenz, C.; Michel, M.S. Experimental models for therapeutic studies of transitional cell carcinoma. *Anticancer. Res.* **2007**, *27*, 3163–3171. [PubMed]

64. Truschel, S.T.; Ruiz, W.G.; Shulman, T.; Pilewski, J.; Sun, T.T.; Zeidel, M.L.; Apodaca, G. Primary uroepithelial cultures. A model system to analyze umbrella cell barrier function. *J. Biol. Chem.* **1999**, *274*, 15020–15029. [CrossRef]

65. Celis, A.; Rasmussen, H.H.; Celis, P.; Basse, B.; Lauridsen, J.B.; Ratz, G.; Hein, B.; Ostergaard, M.; Wolf, H.; Orntoft, T.; et al. Short-term culturing of low-grade superficial bladder transitional cell carcinomas leads to changes in the expression levels of several proteins involved in key cellular activities. *Electrophoresis* **1999**, *20*, 355–361. [CrossRef]

66. Almeida, J.L.; Cole, K.D.; Plant, A.L. Standards for Cell Line Authentication and Beyond. *PLoS Biol.* **2016**, *14*, e1002476. [CrossRef] [PubMed]

67. Parache, R.M.; Droulle, P.; Notter, D.; Vigneron, C. A new method of implanting orthotopic rat bladder tumor for experimental therapies. *Int. J. Cancer* **2002**, *102*, 280–285. [CrossRef]

68. Chade, D.C.; Andrade, P.M.; Borra, R.C.; Leite, K.R.; Andrade, E.; Villanova, F.E.; Srougi, M. Histopathological characterization of a syngeneic orthotopic murine bladder cancer model. *Int. Braz. J. Urol.* **2008**, *34*, 220–229. [CrossRef]

69. Gunther, J.H.; Frambach, M.; Deinert, I.; Brandau, S.; Jocham, D.; Bohle, A. Effects of acetylic salicylic acid and pentoxifylline on the efficacy of intravesical BCG therapy in orthotopic murine bladder cancer (MB49). *J. Urol.* **1999**, *161*, 1702–1706. [CrossRef]

70. Werthman, P.E.; Drazan, K.E.; Rosenthal, J.T.; Khalili, R.; Shaked, A. Adenoviral-p53 gene transfer to orthotopic and peritoneal murine bladder cancer. *J. Urol.* **1996**, *155*, 753–756. [CrossRef]

71. Dobek, G.L.; Godbey, W.T. An Orthotopic Model of Murine Bladder Cancer. *J. Vis. Exp.* **2011**, *48*, 2535. [CrossRef] [PubMed]

72. Soloway, M.S.; Masters, S. Urothelial susceptibility to tumor cell implantation influence of cauterization. *Cancer* **1980**, *46*, 1158–1163. [CrossRef]

73. Yang, X.; Ren, L.; Wang, G.; Zhao, L.; Zhang, H.; Mi, Z.; Bai, X. A New Method of Establishing Orthotopic Bladder Transplantable Tumor in Mice. *Cancer Biol. Med.* **2012**, *9*, 261–265. [PubMed]

74. Soloway, M.S. Intravesical and systemic chemotherapy of murine bladder cancer. *Cancer Res.* **1977**, *37*, 2918–2929.

75. Zhang, Z.; Xu, X.; Zhang, X.; Chen, X.; Chen, Q.; Dong, L.; Hu, Z.; Li, J.; Gao, J. The therapeutic potential of SA-sCD40L in the orthotopic model of superficial bladder cancer. *Acta Oncol.* **2011**, *50*, 1111–1118. [CrossRef]

76. Weldon, T.E.; Soloway, M.S. Susceptibility of urothelium to neoplastic cellular implantation. *Urology* **1975**, *5*, 824–827. [CrossRef]

77. Chan, E.; Patel, A.; Heston, W.; Larchian, W. Mouse orthotopic models for bladder cancer research. *BJU Int.* **2009**, *104*, 1286–1291. [CrossRef]

78. Cheon, J.; Moon, D.G.; Cho, H.Y.; Park, H.S.; Kim, J.J.; Gardner, T.A.; Kao, C. Adenovirus-mediated suicide-gene therapy in an orthotopic murine bladder tumor model. *Int. J. Urol.* **2002**, *9*, 261–267. [CrossRef] [PubMed]

79. Smith, E.B.; Schwartz, M.; Kawamoto, H.; You, X.; Hwang, D.; Liu, H.; Scherr, D.S. Antitumor Effects of Imidazoquinolines in Urothelial Cell Carcinoma of the Bladder. *J. Urol.* **2007**, *177*, 2347–2351. [CrossRef]

80. Talmadge, J.E.; Singh, R.K.; Fidler, I.J.; Raz, A. Murine Models to Evaluate Novel and Conventional Therapeutic Strategies for Cancer. *Am. J. Pathol.* **2007**, *170*, 793–804. [CrossRef]

81. Soloway, M.S.; Martino, C.; Hyatt, C.; Marrone, J.C. Immunogenicity of N-[-4-(5-nitro-2-furyl)-2-thiazolyl]formamide-induced bladder cancer. *Natl. Cancer Inst. Monogr.* **1978**, *49*, 293–300.

82. Summerhayes, I.C.; Franks, L.M. Effects of Donor Age on Neoplastic Transformation of Adult Mouse Bladder Epithelium In Vitro. *J. Natl. Cancer Inst.* **1979**, *62*, 1017–1023. [CrossRef]

83. Fodor, I.; Timiryasova, T.; Denes, B.; Yoshida, J.; Ruckle, H.; Lilly, M. Vaccinia virus mediated p53 gene therapy for bladder cancer in an orthotopic murine model. *J. Urol.* **2005**, *173*, 604–609. [CrossRef] [PubMed]

84. Hanel, E.G.; Xiao, Z.; Wong, K.K.; Lee, P.W.; Britten, R.A.; Moore, R.B. A novel intravesical therapy for superficial bladder cancer in an orthotopic model: Oncolytic reovirus therapy. *J. Urol.* **2004**, *172*, 2018–2022. [CrossRef]

85. Reis, L.O.; Ferreira, U.; Billis, A.; Cagnon, V.; Favaro, W.J. Anti-Angiogenic Effects of the Superantigen Staphylococcal Enterotoxin B and Bacillus Calmette-Guérin Immunotherapy for Nonmuscle Invasive Bladder Cancer. *J. Urol.* **2012**, *187*, 438–445. [CrossRef] [PubMed]

86. Miyazaki, J.; Nishiyama, H.; Yano, I.; Nakaya, A.; Kohama, H.; Kawai, K.; Joraku, A.; Nakamura, T.; Harashima, H.; Akaza, H. The therapeutic effects of R8-liposome-BCG-CWS on BBN-induced rat urinary bladder carcinoma. *Anticancer Res.* **2011**, *31*, 2065–2071.

87. Shen, Z.-J.; Wang, Y.; Ding, G.-Q.; Pan, C.-W.; Zheng, R.-M. Study on enhancement of fibronectin-mediated bacillus Calmette-Guérin attachment to urinary bladder wall in rabbits. *World J. Urol.* **2007**, *25*, 525–529. [CrossRef]

88. Black, P.C.; Dinney, C.P.N. Bladder cancer angiogenesis and metastasis—Translation from murine model to clinical trial. *Cancer Metastasis Rev.* **2007**, *26*, 623–634. [CrossRef]

89. Loskog, A.S.; Fransson, M.E.; Totterman, T.T. AdCD40L gene therapy counteracts T regulatory cells and cures aggressive tumors in an orthotopic bladder cancer model. *Clin Cancer Res.* **2005**, *11*, 8816–8821. [CrossRef]

90. Said, N.; Sanchez-Carbayo, M.; Smith, S.C.; Theodorescu, D. RhoGDI2 suppresses lung metastasis in mice by reducing tumor versican expression and macrophage infiltration. *J. Clin. Investig.* **2012**, *122*, 1503–1518. [CrossRef]

91. Said, N.; Smith, S.; Sanchez-Carbayo, M.; Theodorescu, D. Tumor endothelin-1 enhances metastatic colonization of the lung in mouse xenograft models of bladder cancer. *J. Clin. Investig.* **2011**, *121*, 132–147. [CrossRef]

92. Lodillinsky, C.; Rodriguez, V.; Vauthay, L.; Sandes, E.; Casabé, A.; Eiján, A.M. Novel Invasive Orthotopic Bladder Cancer Model With High Cathepsin B Activity Resembling Human Bladder Cancer. *J. Urol.* **2009**, *182*, 749–755. [CrossRef]

93. Wilmanns, C.; Fan, D.; Obrian, C.; Radinsky, R.; Bucana, C.; Tsan, R.; Fidler, I. Modulation of Doxorubicin Sensitivity and Level of P-Glycoprotein Expression in Human Colon-Carcinoma Cells by Ectopic and Orthotopic Environments in Nude-Mice. *Int. J. Oncol.* **1993**, *3*, 413–422. [CrossRef] [PubMed]

94. Overdevest, J.B.; Thomas, S.; Kristiansen, G.; Hansel, D.E.; Smith, S.C.; Theodorescu, D. CD24 Offers a Therapeutic Target for Control of Bladder Cancer Metastasis Based on a Requirement for Lung Colonization. *Cancer Res.* **2011**, *71*, 3802–3811. [CrossRef]

95. Smith, S.C.; Nicholson, B.; Nitz, M.; Frierson, H.F.; Smolkin, M.; Hampton, G.; El-Rifai, W.; Theodorescu, D. Profiling Bladder Cancer Organ Site-Specific Metastasis Identifies LAMC2 as a Novel Biomarker of Hematogenous Dissemination. *Am. J. Pathol.* **2009**, *174*, 371–379. [CrossRef] [PubMed]

96. Casciari, J.J.; Hollingshead, M.G.; Alley, M.C.; Mayo, J.G.; Malspeis, L.; Miyauchi, S.; Grever, M.R.; Weinstein, J.N. Growth and Chemotherapeutic Response of Cells in a Hollow-Fiber In Vitro Solid Tumor Model. *J. Natl. Cancer Inst.* **1994**, *86*, 1846–1852. [CrossRef] [PubMed]

97. Hall, L.A.; Krauthauser, C.M.; Wexler, R.S.; Hollingshead, M.G.; Slee, A.M.; Kerr, J.S. The hollow fiber assay: Continued characterization with novel approaches. *Anticancer Res.* **2000**, *20*, 903–911.

98. Mi, Q.; Lantvit, D.; Reyes-Lim, E.; Chai, H.; Zhao, W.; Lee, I.-S.; Peraza-Sánchez, S.; Ngassapa, O.; Kardono, L.B.S.; Riswan, S.; et al. Evaluation of the Potential Cancer Chemotherapeutic Efficacy of Natural Product Isolates Employing In Vivo Hollow Fiber Tests1. *J. Nat. Prod.* **2002**, *65*, 842–850. [CrossRef]

99. Morrell, A.; Jayaraman, M.; Nagarajan, M.; Fox, B.M.; Meckley, M.R.; Ioanoviciu, A.; Pommier, Y.; Antony, S.; Hollingshead, M.; Cushman, M. Evaluation of indenoisoquinoline topoisomerase I inhibitors using a hollow fiber assay. *Bioorganic Med. Chem. Lett.* **2006**, *16*, 4395–4399. [CrossRef]

100. Moon, K.H.; Han, B.K.; Jeong, S.J.; Hong, S.K.; Byun, S.-S.; Lee, S.E. In Vivo Hollow Fiber Assay for Anticancer Drugs' Responsiveness in a Bladder Cancer Model. *Korean J. Urol.* **2008**, *49*, 392–397. [CrossRef]

101. de Visser, K.E.; Eichten, A.; Coussens, L.M. Paradoxical roles of the immune system during cancer development. *Nat. Rev. Cancer* **2006**, *6*, 24–37. [CrossRef]

102. Mitra, A.P.; Cote, R.J. Molecular Pathogenesis and Diagnostics of Bladder Cancer. *Annu. Rev. Pathol. Mech. Dis.* **2009**, *4*, 251–285. [CrossRef] [PubMed]

103. Knowles, M.A. Molecular pathogenesis of bladder cancer. *Int. J. Clin. Oncol.* **2008**, *13*, 287–297. [CrossRef] [PubMed]

104. Goebell, P.J.; Knowles, M.A. Bladder cancer or bladder cancers? Genetically distinct malignant conditions of the urothelium. *Urol. Oncol. Semin. Orig. Investig.* **2010**, *28*, 409–428. [CrossRef]

105. Esrig, D.; Elmajian, D.; Groshen, S.; Freeman, J.A.; Stein, J.P.; Chen, S.-C.; Nichols, P.W.; Skinner, D.G.; Jones, P.A.; Cote, R.J. Accumulation of nuclear p53 and tumor progression in bladder cancer. *N. Engl. J. Med.* **1994**, *331*, 1259–1264. [CrossRef] [PubMed]

106. Dyrskjøt, L.; Thykjaer, T.; Kruhøffer, M.; Jensen, J.L.; Marcussen, N.; Hamilton-Dutoit, S.; Wolf, H.; Ørntoft, T.F. Identifying distinct classes of bladder carcinoma using microarrays. *Nat. Genet.* **2002**, *33*, 90–96. [CrossRef]

107. Sanchez-Carbayo, M.; Socci, N.D.; Lozano, J.J.; Li, W.; Charytonowicz, E.; Belbin, T.J.; Prystowsky, M.B.; Ortiz, A.R.; Childs, G.; Cordon-Cardo, C. Gene Discovery in Bladder Cancer Progression using cDNA Microarrays. *Am. J. Pathol.* **2003**, *163*, 505–516. [CrossRef]

108. Blaveri, E.; Brewer, J.L.; Roydasgupta, R.; Fridlyand, J.; Devries, S.; Koppie, T.; Pejavar, S.; Mehta, K.; Carroll, P.; Simko, J.P.; et al. Bladder Cancer Stage and Outcome by Array-Based Comparative Genomic Hybridization. *Clin. Cancer Res.* **2005**, *11*, 7012–7022. [CrossRef] [PubMed]

109. Kim, J.-H.; Tuziak, T.; Hu, L.; Wang, Z.; Bondaruk, J.; Kim, M.; Fuller, G.; Dinney, C.; Grossman, H.B.; Baggerly, K.; et al. Alterations in transcription clusters underlie development of bladder cancer along papillary and nonpapillary pathways. *Lab. Investig.* **2005**, *85*, 532–549. [CrossRef]

110. Mitra, A.P.; Pagliarulo, V.; Yang, D.; Waldman, F.M.; Datar, R.H.; Skinner, D.G.; Groshen, S.; Cote, R.J. Generation of a Concise Gene Panel for Outcome Prediction in Urinary Bladder Cancer. *J. Clin. Oncol.* **2009**, *27*, 3929–3937. [CrossRef]

111. Riester, M.; Taylor, J.M.; Feifer, A.; Koppie, T.; Rosenberg, J.; Downey, R.J.; Bochner, B.; Michor, F. Combination of a Novel Gene Expression Signature with a Clinical Nomogram Improves the Prediction of Survival in High-Risk Bladder Cancer. *Clin. Cancer Res.* **2012**, *18*, 1323–1333. [CrossRef] [PubMed]

112. Lauss, M.; Ringnér, M.; Höglund, M. Prediction of Stage, Grade, and Survival in Bladder Cancer Using Genome-wide Expression Data: A Validation Study. *Clin. Cancer Res.* **2010**, *16*, 4421–4433. [CrossRef] [PubMed]

113. Lindgren, D.; Frigyesi, A.; Gudjonsson, S.; Sjödahl, G.; Hallden, C.; Chebil, G.; Veerla, S.; Ryden, T.; Månsson, W.; Liedberg, F.; et al. Combined Gene Expression and Genomic Profiling Define Two Intrinsic Molecular Subtypes of Urothelial Carcinoma and Gene Signatures for Molecular Grading and Outcome. *Cancer Res.* **2010**, *70*, 3463–3472. [CrossRef]

114. Zieger, K.; Marcussen, N.; Borre, M.; Ørntoft, T.F.; Dyrskjøt, L. Consistent genomic alterations in carcinoma in situ of the urinary bladder confirm the presence of two major pathways in bladder cancer development. *Int. J. Cancer* **2009**, *125*, 2095–2103. [CrossRef] [PubMed]

115. Hurst, C.D.; Platt, F.M.; Taylor, C.F.; Knowles, M.A. Novel Tumor Subgroups of Urothelial Carcinoma of the Bladder Defined by Integrated Genomic Analysis. *Clin. Cancer Res.* **2012**, *18*, 5865–5877. [CrossRef]

116. Lindgren, D.; Sjödahl, G.; Lauss, M.; Staaf, J.; Chebil, G.; Lövgren, K.; Gudjonsson, S.; Liedberg, F.; Patschan, O.; Månsson, W.; et al. Integrated Genomic and Gene Expression Profiling Identifies Two Major Genomic Circuits in Urothelial Carcinoma. *PLoS ONE* **2012**, *7*, e38863. [CrossRef]

117. Sjodahl, G.; Lauss, M.; Lövgren, K.; Chebil, G.; Gudjonsson, S.; Veerla, S.; Patschan, O.; Aine, M.; Fernö, M.; Ringnér, M.; et al. A molecular taxonomy for urothelial carcinoma. *Clin. Cancer Res.* **2012**, *18*, 3377–3386. [CrossRef] [PubMed]

118. Nordentoft, I.; Lamy, P.; Birkenkamp-Demtröder, K.; Shumansky, K.; Vang, S.; Hornshøj, H.; Juul, M.; Villesen, P.; Hedegaard, J.; Roth, A.; et al. Mutational Context and Diverse Clonal Development in Early and Late Bladder Cancer. *Cell Rep.* **2014**, *7*, 1649–1663. [CrossRef]

119. Pan, C.-X.; Zhang, H.; Tepper, C.G.; Lin, T.-Y.; Davis, R.; Keck, J.; Ghosh, P.M.; Gill, P.; Airhart, S.; Bult, C.; et al. Development and Characterization of Bladder Cancer Patient-Derived Xenografts for Molecularly Guided Targeted Therapy. *PLoS ONE* **2015**, *10*, e0134346. [CrossRef]

120. Chan, K.S.; Espinosa, I.; Chao, M.; Wong, D.; Ailles, L.; Diehn, M.; Gill, H.; Presti, J.; Chang, H.Y.; van de Rijn, M.; et al. Identification, molecular characterization, clinical prognosis, and therapeutic targeting of human bladder tumor-initiating cells. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 14016–14021. [CrossRef]

121. Kurtova, A.V.; Xiao, J.; Mo, Q.; Pazhanisamy, S.K.; Krasnow, R.; Lerner, S.P.; Chen, F.; Roh, T.; Lay, E.; Ho, P.L.; et al. Blocking PGE2-induced tumour repopulation abrogates bladder cancer chemoresistance. *Nat. Cell Biol.* **2015**, *517*, 209–213. [CrossRef]

122. Suh, Y.S.; Jeong, K.-C.; Lee, S.-J.; Seo, H.K. Establishment and application of bladder cancer patient-derived xenografts as a novel preclinical platform. *Transl. Cancer Res.* **2017**, *6*, S733–S743. [CrossRef]

123. Tentler, J.J.; Tan, A.C.; Weekes, C.D.; Jimeno, A.; Leong, S.; Pitts, T.M.; Arcaroli, J.J.; Messersmith, W.A.; Eckhardt, S.G. Patient-derived tumour xenografts as models for oncology drug development. *Nat. Rev. Clin. Oncol.* **2012**, *9*, 338–350. [CrossRef]

124. Kerbel, R.S. Human tumor xenografts as predictive preclinical models for anticancer drug activity in humans: Better than commonly perceived-but they can be improved. *Cancer Biol. Ther.* **2003**, *2*, 133–138. [CrossRef]

125. Shorthouse, A.J.; Smyth, J.F.; Steel, G.G.; Ellison, M.; Mills, J.; Peckham, M.J. The human tumour xenograft—A valid model in experimental chemotherapy? *Br. J. Surg.* **2005**, *67*, 715–722. [CrossRef] [PubMed]

126. Shimosato, Y.; Kameya, T.; Nagai, K.; Hirohashi, S.; Koide, T.; Hayashi, H.; Nomura, T. Transplantation of Human Tumors in Nude Mice23. *J. Natl. Cancer Inst.* **1976**, *56*, 1251–1260. [CrossRef]

127. Brehm, M.A.; Cuthbert, A.; Yang, C.; Miller, D.M.; DiIorio, P.; Laning, J.; Burzenski, L.; Gott, B.; Foreman, O.; Kavirayani, A. Parameters for establishing humanized mouse models to study human immunity: Analysis of human hematopoietic stem cell engraftment in three immunodeficient strains of mice bearing the IL2rγ^{null} mutation. *Clin. Immunol.* **2010**, *135*, 84–98. [CrossRef]

128. Ruddell, A.; Mezquita, P.; Brandvold, K.A.; Farr, A.; Iritani, B.M. B Lymphocyte-Specific c-Myc Expression Stimulates Early and Functional Expansion of the Vasculature and Lymphatics during Lymphomagenesis. *Am. J. Pathol.* **2003**, *163*, 2233–2245. [CrossRef]

129. Qian, B.-Z.; Pollard, J.W. Macrophage Diversity Enhances Tumor Progression and Metastasis. *Cell* **2010**, *141*, 39–51. [CrossRef]

130. Park, B.; Jeong, B.C.; Choi, Y.-L.; Kwon, G.Y.; Lim, J.E.; Seo, S.I.; Jeon, S.S.; Lee, H.M.; Choi, H.Y.; Lee, K.-S. Development and characterization of a bladder cancer xenograft model using patient-derived tumor tissue. *Cancer Sci.* **2013**, *104*, 631–638. [CrossRef]

131. Cirone, P.; Andresen, C.J.; Eswaraka, J.R.; Lappin, P.B.; Bagi, C.M. Patient-derived xenografts reveal limits to PI$_3$K/mTOR- and MEK-mediated inhibition of bladder cancer. *Cancer Chemother. Pharmacol.* **2014**, *73*, 525–538. [CrossRef] [PubMed]

132. Bolenz, C.; Wenzel, M.; Cao, Y.; Trojan, L.; Häcker, A.; Arancibia, M.F.; Alken, P.; Michel, M.S. Newly Developed Mini-Endoscope for Diagnosis and Follow-Up of Orthotopic Bladder Transitional-Cell Carcinoma In Vivo. *J. Endourol.* **2007**, *21*, 789–794. [CrossRef]

133. Zlatev, D.V.; Altobelli, E.; Liao, J.C. Advances in Imaging Technologies in the Evaluation of High-Grade Bladder Cancer. *Urol. Clin. N. Am.* **2015**, *42*, 147–157. [CrossRef]

134. Kikuchi, E.; Xu, S.; Ohori, M.; Matei, C.; Lupu, M.; Menendez, S.; Koutcher, J.A.; Bochner, B.H. Detection and Quantitative Analysis of Early Stage Orthotopic Murine Bladder Tumor Using In Vivo Magnetic Resonance Imaging. *J. Urol.* **2003**, *170*, 1375–1378. [CrossRef] [PubMed]

135. Satoh, H.; Morimoto, Y.; Arai, T.; Asanuma, H.; Kawauchi, S.; Seguchi, K.; Kikuchi, M.; Murai, M. Intravesical Ultrasonography for Tumor Staging in an Orthotopically Implanted Rat Model of Bladder Cancer. *J. Urol.* **2007**, *177*, 1169–1173. [CrossRef]

136. Foster, W.K.; Ford, N.L. Investigating the effect of longitudinal micro-CT imaging on tumour growth in mice. *Phys. Med. Biol.* **2010**, *56*, 315–326. [CrossRef] [PubMed]

137. Marcu, R.D.; Diaconu, C.C.; Constantin, T.; Socea, B.; Ionita-Radu, F.; Mischianu, D.L.D.; Bratu, O.G. Minimally invasive biopsy in retroperitoneal tumors (Review). *Exp. Ther. Med.* **2019**, *18*, 5016–5020. [CrossRef]

138. Kolkman, R.G.M.; Brands, P.J.; Steenbergen, W.; van Leeuwen, T. Real-Time in vivo photoacoustic and ultrasound imaging. *J. Biomed. Opt.* **2008**, *13*, 050510. [CrossRef]

139. Keyaerts, M.; Caveliers, V.; Lahoutte, T. Bioluminescence imaging: Looking beyond the light. *Trends Mol. Med.* **2012**, *18*, 164–172. [CrossRef]

140. Iorga, R.A.; Bratu, O.G.; Marcu, R.D.; Constantin, T.; Mischianu, D.L.D.; Socea, B.; Găman, M.-A.; Diaconu, C.C. Venous thromboembolism in cancer patients: Still looking for answers (Review). *Exp. Ther. Med.* **2019**, *18*, 5026–5032. [CrossRef]