Jurnal Abdi Kesehatan dan Kedokteran (JAKK)
p-ISSN: 2962-8245 | e-ISSN: 2962-7133
Vol. 5, No. 1, Januari 2026

Original Article

Fibroblas Terkait Kanker dan Dampaknya terhadap Kelangsungan Hidup Keseluruhan pada Karsinoma Urotelial Kandung Kemih: Suatu Tinjauan Sistematis

Cancer-Associated Fibroblasts and Their Impact on Overall Survival in Bladder Urothelial Carcinoma: A Systematic Review

Vico Mardenanta[1], Jodii Arlan Kurnia[2,3*]

[1] *General Practitioner, Bhayangkara H.S. Samsoeri Mertojoso Hospital, Surabaya, East Java, Indonesia*
[2] *Head of Casemix, Abdhi Family General Hospital, Banyuwangi, East Java, Indonesia*
[3] *Magister Management of Hospital, Medical Faculty, University Of Brawijaya, Malang, East Java, Indonesia*

***Corresponding Email**: jodiarlan@gmail.com*

ABSTRACT

Background: Bladder urothelial carcinoma (BUC) remains a common urologic malignancy with substantial mortality. The tumor microenvironment (TME), particularly cancer-associated fibroblasts (CAFs), promotes extracellular matrix remodeling, angiogenesis, immune evasion, and treatment resistance, suggesting CAFs may serve as prognostic and predictive biomarkers. Objective: To evaluate the association between CAFs and overall survival (OS) in BUC and identify clinically relevant CAF-related markers.

Methods: A PRISMA-guided systematic review was performed using PubMed, Cochrane Library, Taylor & Francis, ProQuest, Scopus, and EMBASE (2015–2025). Eligible human studies assessing CAFs/CAF-signatures in BUC were included. Risk of bias was assessed using ROBINS-I.

Results: Nine studies were included. Across cohorts, elevated CAF abundance or CAF-related markers consistently correlated with poorer outcomes. High CAF infiltration in TCGA-BLCA was associated with reduced OS (p=0.003) and advanced stage (p<0.001). Stromal markers such as FAP (HR=2.06) and PDGFRβ (HR=1.75) predicted worse OS, with FAP-dominant phenotypes showing the lowest survival. myCAF- and iCAF-high subtypes were linked to shorter OS (e.g., iCAF cluster p=0.024). Multiple CAF-based gene signatures (e.g., 7-gene models) stratified mortality risk (multivariate HR up to 3.51; AUC 0.88). COL10A1 overexpression also predicted inferior OS. A fibroblast-related gene index (FRGI) showed strong performance in ICI-treated patients (1-year OS AUC=0.95) and was enriched in non-responders.

Conclusion: CAF abundance and subtype composition are robust adverse prognostic indicators in BUC and show promise for risk stratification and treatment decision support, particularly for immunotherapy responsiveness.

Keywords: *Cancer-associated fibroblasts, CAF, bladder urothelial carcinoma, tumor microenvironment, prognosis, survival*

ABSTRAK

Latar belakang: Bladder urothelial carcinoma (BUC) merupakan keganasan urologi yang sering ditemukan dengan mortalitas tinggi. Tumor microenvironment (TME), khususnya cancer-associated fibroblasts (CAFs), berperan dalam extracellular matrix remodeling, angiogenesis, immune evasion, serta treatment resistance, sehingga berpotensi menjadi biomarker prognostic dan predictive. Tujuan : Menilai hubungan CAFs dengan overall survival (OS) pada BUC dan mengidentifikasi CAF-related markers yang relevan secara klinis.

Metode: Systematic review mengikuti PRISMA dilakukan pada PubMed, Cochrane Library, Taylor & Francis, ProQuest, Scopus, dan EMBASE (2015–2025). Studi pada manusia yang menilai CAFs/CAF-signatures pada BUC diikutsertakan. Risk of bias dinilai menggunakan ROBINS-I.

Hasil: Sembilan studi memenuhi kriteria. Secara konsisten, peningkatan CAF abundance atau CAF-related markers berhubungan dengan luaran yang lebih buruk. High CAF infiltration pada TCGA-BLCA berkaitan dengan penurunan OS (p=0,003) dan advanced stage (p<0,001). Stromal markers seperti FAP (HR=2,06) dan PDGFRβ (HR=1,75) memprediksi OS yang lebih rendah, dengan FAP-dominant phenotypes menunjukkan survival terendah. Subtypes myCAF-high dan iCAF-high juga terkait OS lebih pendek (misalnya iCAF cluster p=0,024). Beberapa CAF-based gene signatures (mis. 7-gene model) mampu menstratifikasi mortality risk (multivariate HR hingga 3,51; AUC 0,88). COL10A1 overexpression turut memprediksi OS yang lebih buruk. Fibroblast-related gene index (FRGI) menunjukkan performa tinggi pada ICI-treated patients (1-year OS AUC=0,95) dan lebih sering pada non-responders.

Kesimpulan: CAF abundance dan CAF subtype composition merupakan adverse prognostic indicators yang kuat pada BUC serta berpotensi mendukung risk stratification dan treatment decision support, terutama terkait immunotherapy responsiveness.

Kata kunci: cancer-associated fibroblasts, CAF, bladder urothelial carcinoma, tumor microenvironment, prognosis, survival

Submit: November 1, 2025| ***Accepted***: January 29, 2026| ***Online***: January 31, 2026
Citation: Kurnia, J. A., & Mardenanta, V. (2026). Fibroblas Terkait Kanker dan Dampaknya terhadap Kelangsungan Hidup Keseluruhan pada Karsinoma Urotelial Kandung Kemih: Suatu Tinjauan Sistematis: Cancer-Associated Fibroblasts and Their Impact on Overall Survival in Bladder Urothelial Carcinoma: A Systematic Review. Jurnal Abdi Kesehatan Dan Kedokteran, 5(1), 441–450.
https://doi.org/10.55018/jakk.v5i1.166

Key Findings

- ⇒ Higher CAF abundance and specific CAF subtypes (myCAF-high and iCAF-high) are consistently associated with reduced overall survival and more advanced disease in bladder urothelial carcinoma.
- ⇒ Stromal markers and CAF-related gene signatures, including FAP, PDGFRβ, COL10A1, and multi-gene models, effectively stratify mortality risk and predict poorer survival outcomes.
- ⇒ Fibroblast-based indices such as the fibroblast-related gene index demonstrate strong prognostic and predictive value, particularly in identifying non-responders to immune checkpoint inhibitor therapy.

Introduction

Bladder urothelial carcinoma (BUC) remains one of the most lethal urogenital malignancies worldwide, with approximately 573,278 new cases and 212,536 deaths reported in 2020 (Wang et al, 2023). The disease presents as non-muscle-invasive bladder cancer (NMIBC), muscle-invasive bladder cancer (MIBC), and metastatic disease, with MIBC representing approximately 25% of patients at the time of diagnosis, yet being associated with 5-year overall survival rates of approximately 50% (Sung et al., 2021 ; Koll et al., 2023). Despite significant advances in treatment modalities, including radical cystectomy and platin-based chemotherapy for MIBC, and immune checkpoint inhibitors for advanced disease, these therapeutic approaches remain limited with incomplete response rates and considerable toxicity (Bellmunt et al., 2021). Therefore, the identification of novel prognostic and predictive biomarkers is critical for optimizing patient outcomes and implementing personalized treatment strategies in bladder cancer management (Bellmunt et al., 2021).

The tumor microenvironment (TME) plays a pivotal role in bladder cancer development, tumor cell invasion, metastatic dissemination, and therapeutic responsiveness (Burley et al., 2022). The TME comprises various cellular and acellular components including tumor cells, immune cells, stromal cells, vasculature, and extracellular matrix (ECM) (Wang et al., 2023). Among the stromal components, cancer-associated fibroblasts (CAFs) represent key cellular populations within the TME and are involved in multiple aspects of tumor biology, including ECM remodeling, angiogenesis, epithelial-mesenchymal transition (EMT), and immune evasion. Evidence demonstrates that CAFs profoundly influence patient prognosis and response to both chemotherapy and immunotherapy. Furthermore, CAF-derived factors modulate the immune microenvironment through recruitment and polarization of immunosuppressive cell populations, including regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) (Burley et al., 2022 ; Chen et al., 2022).

The extracellular matrix (ECM), a major component of the tumor stroma, plays a vital role in tumor establishment, disease progression, and modulating therapeutic efficacy. Collagen, the primary structural protein of the ECM that participates in cancer fibrosis, influences cancer cell behavior and can be reversely reshaped by cancer cells to promote progression. Numerous studies have identified collagen as a prognostic factor and demonstrated associations with resistance to chemotherapy and targeted drugs in various cancers (Wang et al., 2023). The tumor-stroma ratio (TSR), which quantifies the proportion of stromal components relative to tumor cells, has emerged as an important prognostic parameter. High stromal content has been shown to correlate with poor survival in diverse tumor types, including MIBC. Recent evidence indicates that TSR-based classification may provide valuable prognostic information and guide therapeutic decision-making in MIBC patients (Liu et al., 2022) .

Tumor budding (TB) represents another important histological feature reflecting aggressive tumor behavior and invasive potential at the tumor-stromal interface. The combination of TSR and TB assessment provides a comprehensive morphological evaluation that may better predict patient outcomes than individual parameters alone. Furthermore, emerging single-cell RNA sequencing (scRNA-seq) technologies have revealed significant heterogeneity within the CAF population, identifying distinct subtypes including myofibroblastic CAFs (myCAFs) and inflammatory CAFs (iCAFs) with distinct biological properties and prognostic implications (Liu et al., 2022).

CAF heterogeneity is increasingly recognized as fundamental to understanding tumor progression and therapeutic response (Chen et al, 2022). Different CAF subtypes exhibit distinct functional characteristics, with myCAFs predominantly involved in ECM deposition and structural support, while iCAFs are primarily characterized by enhanced secretion of inflammatory cytokines and immunomodulatory factors. Recent investigations have identified specific CAF markers

including fibroblast activation protein (FAP), alpha-smooth muscle actin (α-SMA or ACTA2), CD90, and platelet-derived growth factor receptors (PDGFRα and PDGFRβ), each associated with distinct biological functions and prognostic significance. Moreover, emerging evidence suggests that specific CAF subtypes, particularly LOXL2-expressing inflammatory CAFs (LOXL2+ iCAFs), play critical roles in promoting tumor progression through IL-32 mediated signaling pathways and M2 macrophage polarization (Burley et al., 2022 ; Chen et al., 2022).

The relationship between CAF characteristics and immune microenvironment composition has become increasingly apparent. High CAF infiltration, particularly in stroma-predominant tumors, has been associated with reduced CD8+ T-cell infiltration and impaired anti-tumor immune responses. Conversely, CAF-enriched tumors demonstrate elevated frequencies of immunosuppressive immune cells, including M2-polarized macrophages and regulatory T cells, which collectively create an immune-excluded phenotype resistant to checkpoint inhibitor therapy. These immune characteristics are further supported by the expression of inhibitory checkpoint molecules such as PD-L1, PD-1, CTLA-4, and LAG-3, which are preferentially upregulated in CAF-rich tumors (Burley et al., 2022 ; Koll et al., 2023). However, the strength and consistency of these associations in bladder urothelial carcinoma remain unclear due to heterogeneity in CAF markers and immune-cell quantification across studies, limiting their translation into clear prognostic or predictive biomarkers. Therefore, this systematic review synthesizes available evidence linking CAF features with immune contexture and therapy response to identify clinically actionable CAF–immune signatures.

Tumor-associated macrophages (TAMs), particularly M2-polarized macrophages, represent a central component of the CAF-rich tumor microenvironment. M2 macrophages are contributors to pro-tumor and anti-inflammatory activity and have been identified as independent prognostic factors in MIBC, with high TAM infiltration associated with poor patient outcomes even in the context of adjuvant chemotherapy. Interestingly, regulatory T cells (Tregs), identified by FoxP3 expression, have shown paradoxical prognostic significance, with high Treg infiltration sometimes representing a marker of heightened overall immune infiltration rather than pure immunosuppression. The complex interplay between these immune populations and stromal fibroblasts significantly influences both tumor progression and therapeutic response (Koll et al., 2023).

The role of CAFs in therapeutic resistance has been extensively documented across multiple cancer types. CAFs promote cisplatin resistance in bladder cancer cells through multiple mechanisms including increased IGF-1/ERβ/Bcl-2 signaling and TGF-β-dependent epithelial-mesenchymal transition (EMT). Additionally, CAF-mediated remodeling of the ECM creates a densely crosslinked matrix that acts as a physical barrier to restrict immune cell penetration and chemotherapeutic drug delivery to tumor cells. This dense stromal architecture, particularly in "stroma-rich" molecular subtypes, has been associated with poor response to neo-adjuvant chemotherapy.

Furthermore, accumulating evidence suggests that CAFs play a critical role in creating an immune-excluded phenotype by promoting the deposition of collagen and other ECM components that physically trap infiltrating T cells in peritumoral regions, preventing them from reaching and eliminating tumor cells (Burley et al., 2022 ; Liu et al., 2022).

Despite extensive research into CAF biology in multiple cancer types, research into CAF characteristics and their functional implications specifically in bladder cancer has been relatively understudied compared to other common malignancies such as pancreatic and breast cancer. The complex relationship between CAF characteristics, tumor-stroma ratio, CAF heterogeneity (particularly specific CAF subtypes like LOXL2+ iCAFs), immune microenvironment composition, and clinical outcomes in bladder urothelial carcinoma remains incompletely understood. Understanding these relationships is essential for developing optimized targeted therapeutic strategies and identifying patients most likely to benefit from specific treatment modalities (Burley et al., 2022).

This systematic review aims to comprehensively evaluate the role of cancer-associated fibroblasts and tumor microenvironment characteristics in influencing prognosis and therapeutic response in patients with bladder urothelial carcinoma, and to identify CAF-related markers as potential prognostic and predictive biomarkers for clinical implementation.

Methods

Design, Participants, and Setting

This systematic review was conducted on 7 December 2025 in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (Page et al., 2021). A comprehensive literature search was performed across multiple databases including PubMed, the Cochrane Library, Taylor & Francis, ProQuest, Scopus, and EMBASE. The search strategy incorporated combinations of keywords related: ("bladder cancer" OR "urothelial carcinoma" OR "bladder urothelial carcinoma" OR "BLCA" OR "bladder neoplasm") AND ("tumor microenvironment" OR "TME" OR "stromal" OR "stroma" OR "cancer-associated fibroblasts" OR "CAFs" OR "extracellular matrix" OR "ECM" OR "tumor-stroma interaction" OR "stromal remodeling" OR "tumor infiltrating immune cells" OR "TIICs" OR "immune microenvironment") AND ("progression" OR "invasion" OR "metastasis" OR "prognosis" OR "survival").

Inclusion and Exclusion Criteria

Studies were eligible for inclusion if they met all of the following criteria: (1) published in English-language journals indexed in Scopus databases with Q1-Q4 ratings; (2) published between 2015 and 2025; (3) examined cancer-associated fibroblasts (CAFs) in bladder urothelial carcinoma; (4) employed study designs including retrospective cohort studies, prospective cohort studies, case-control studies, experimental studies, or bioinformatics analyses with human data; (5) contained clinicopathological data

compatible with PICO framework parameters. Studies were excluded if they were: (1) in vitro or in vivo animal model studies without clinical correlation; (2) review articles, narrative reviews, editorials, or opinion pieces; (3) case reports or small case series (n<5); (4) investigations focused exclusively on metastatic disease without primary tumor analysis; or (5) publications with incomplete data or unavailable full texts.

PICO Framework

The research question was structured using the Population, Intervention/Exposure, Comparison, and Outcome (PICO) framework:

- Population (P): Patients with Bladder urothelial carcinoma
- Intervention/Exposure (I): High infiltration of specific CAF subtype.
- Comparison (C): Low infiltration of the same CAF subtype.
- Outcomes (O): Overall survival (OS).

Study Selection Process

Reviewer screened all identified citations in a two-stage process. In the first stage, titles and abstracts were reviewed for relevance using predetermined inclusion criteria. In the second stage, full-text articles of potentially eligible studies were obtained reviewed used the Rayyan intelligent systematic review website (https://www.rayyan.ai) The complete study selection process was documented using a PRISMA flow diagram. The author conducted a risk of bias assessment using a Risk of Bias In Non-randomized Studies - of Interventions (ROBINS-I) criteria. This list evaluated the bias due to confounding, bias due selection of participants, bias in classification of interventions, bias due to deviations from intended interventions, bias due to missing data, bias in measurement of outcomes, bias in selection of the reported result.

Results

Study Selection and Characteristics

Selection In this study, we conducted a systematic search across multiple databases, including PubMed, Cochrane, Taylor & Francis, Proquest, Scopus, and citation searching, to identify potential papers. The PRISMA flow diagram visually represents the systematic process of selecting studies for review. Of the 2,199 records initially identified from various databases, 243 were identified as duplicates and subsequently removed. After screening 1,956 records, 1,857 were excluded because they did not meet the inclusion criteria. Of the 99 reports sought for retrieval, 74 could not be recovered. During the eligibility assessment, 66 reports were excluded due to irretrievability, data extraction problems, and incompatible language. Ultimately, nine studies were included in the review, demonstrating a rigorous selection process to ensure the relevance and quality of the studies (**Figure 1**).

Risk of BIAS Assessment

Several included studies qualities were assessed and evaluated across seven domains of bias: confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of the reported result. Each domain is color-coded, with green indicating a low risk of bias and yellow indicating a moderate risk. The overall

bias is also assessed. Studies from Du et al., 2021 and Muilwijk et al., 2021 show moderate bias risk due to cofounding and the selection of participant. Despite these specific concerns, all other domains for these studies and all domains for the remaining studies are rated as low risk. Overall, the studies demonstrate a low risk of bias, indicating that their results are reliable. (**Figure 2**).

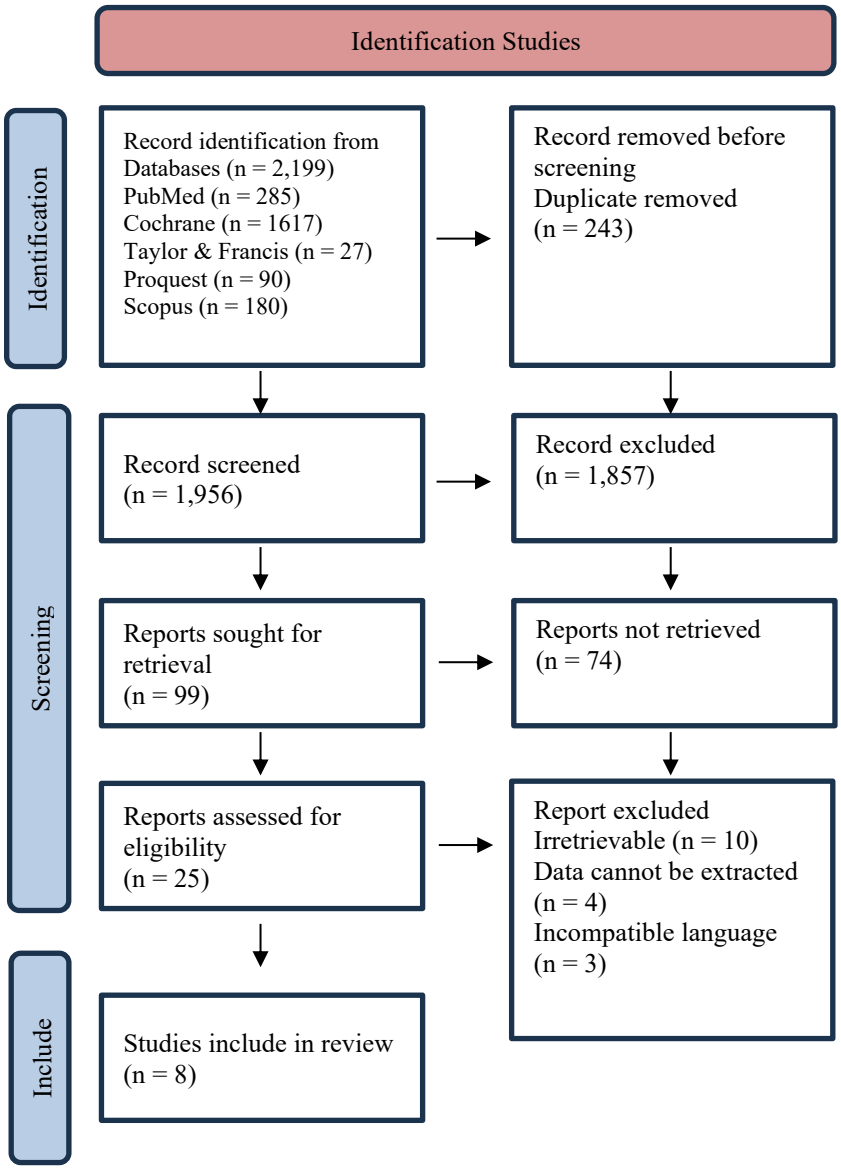

Figure 1. Preferred reporting items for systematic review and meta-analysis (PRISMA) flow diagram of the literature search process in this systematic review

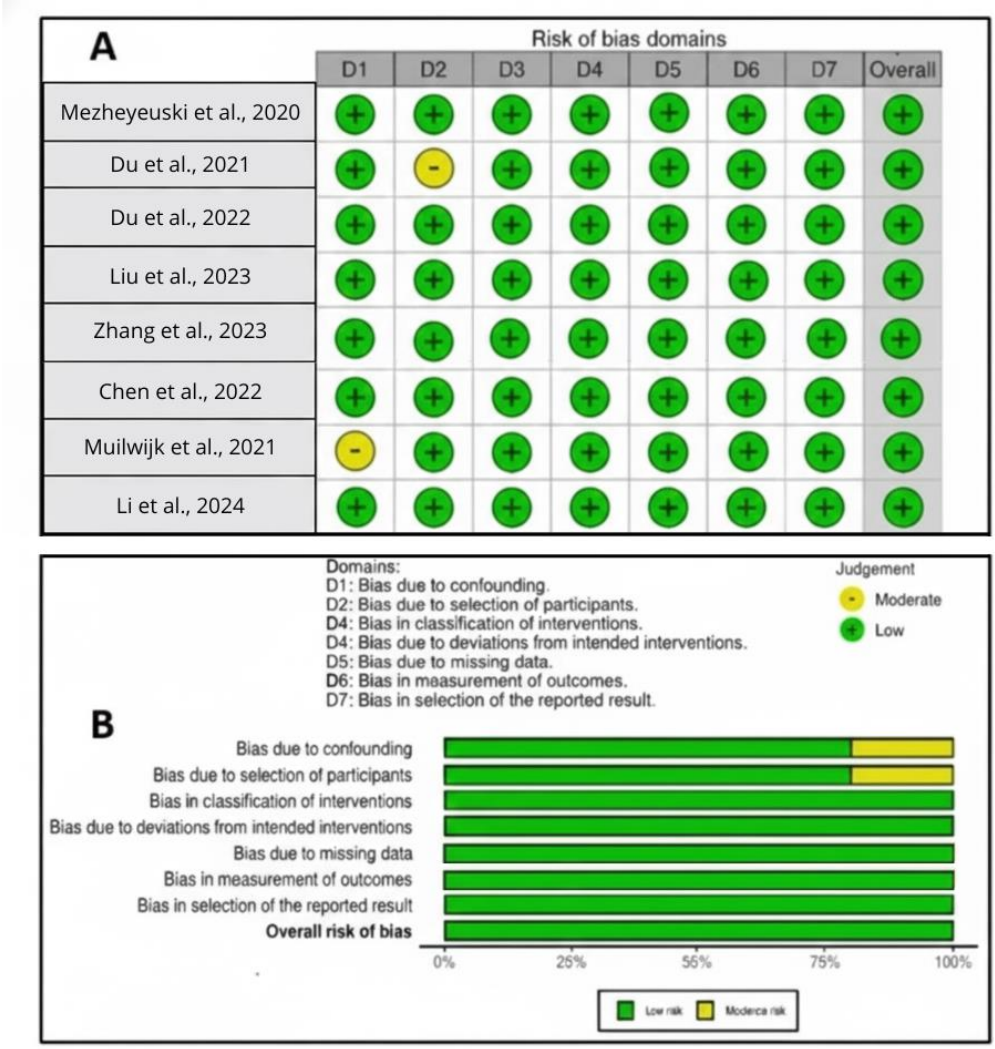

Figure 2. Risk of Bias Assessment. (A) Graphic Risk of Bias, (B) Summary Risk of Bias

Study Characteristics

Table 1.The results from nine included studies are presented.

Author	Study Design	Sample Size	Objective	Grade Cancer Studied
Liu et al, 2023	Retrospective multiomics study	>400 sample	To construct a CAF-based risk signature for predicting prognosis, immune landscape stratification, and treatment selection in bladder urothelial carcinoma patients	All grades; focus on muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC)
Muilwijk et al, 2021	Retrospective cohort study	86 sample	To determine the prognostic biomarker potential of FAP expression in predicting progression, recurrence-free survival, progression-free survival, cancer-specific survival, and overall survival in HG T1 NMIBC patients	High grade (HG) T1 non-muscle invasive bladder cancer (NMIBC)
Mezheyeuski et al, 2020	Retrospective cohort study	344 sample	To investigate associations between fibroblast marker phenotypes and urothelial bladder cancer patient survival, and to identify prognostic patient subgroups based on stromal marker combinations and CD8 T cell status	All grades including Ta (non-invasive), T1 (lamina propria invasive), T2-T4 (muscle-invasive); both high-grade and low-grade tumors

Author	Study Design	Sample Size	Objective	Grade Cancer Studied
Du et al, 2021	Retrospective bioinformatics study	430 sample	To identify and validate CALD1 as a prognostic biomarker for bladder cancer progression and prognosis through bioinformatics analysis and establish its correlation with immune infiltration and tumor microenvironment composition	All grades including high-grade and low-grade; TNM stages I-IV (T1-T4, N0-N1, M0-M1)
Chen et al, 2022	Comprehensive retrospective bioinformatics study	> 500 sample	To identify inflammatory CAF subtypes associated with poor prognosis and treatment resistance, develop an iCAF-based signature for predicting prognosis and immunotherapy response, and validate LOXL2 as a therapeutic target in bladder cancer	All grades; NMIBC (non-muscle invasive) and MIBC (muscle-invasive) categories; analysis includes various tumor stages and grades
Li et al, 2024	Comprehensive retrospective bioinformatics study	> 500 sample	To establish a machine learning-derived fibroblast-related gene index (FRGI) and CD8-FRG molecular subtypes for predicting prognosis and immunotherapy response in BLCA, especially muscle-invasive bladder cancer	All grades and stages; focus on muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC); various TNM stages
Du et al, 2022	Comprehensive retrospective bioinformatic	406 sample	To investigate the bimodal roles of myofibroblasts in	All grades and stages; analysis includes both

Author	Study Design	Sample Size	Objective	Grade Cancer Studied
	s study		bladder cancer development, including protective roles in carcinogenesis and pro-tumoral roles in progression, and to identify myCAF-based biomarkers for prognosis and treatment responsiveness	NMIBC and MIBC; covers the progression from normal tissues through carcinogenesis to advanced disease
Zhang et al, 2023	Comprehensive retrospective bioinformatics study	958 sample	To construct and validate a five-gene CAF-related prognostic model for stratifying bladder cancer patients, assess immune microenvironment composition differences between CAF subtypes, and determine tumor mutational burden and chemotherapy drug sensitivity	All grades and stages; analysis includes various tumor grades, TNM stages, and classification into high-risk and low-risk groups based on CAF-related gene expression

Table 2. Results type of CAF and association with Overall Survival.

Author	Type of CAF Studied	Patient Population	Association with OS	CAF Assessment Method
(Mezheyeuski et al., 2020)	General CAFs; fibroblast subtypes defined by α-SMA, CD90, FAP, PDGFRα/β	Urothelial bladder carcinoma (NMIBC and MIBC)	FAP-dominant stromal phenotype was independently associated with significantly worse 5-year OS	Immunohistochemistry (IHC) using multiple fibroblast markers; cluster analysis of stromal phenotypes
(Du et al.,	General CAFs	Bladder	High CALD1	CAF abundance

Author	Type of CAF Studied	Patient Population	Association with OS	CAF Assessment Method
2021)	(CAF-related gene CALD1)	cancer (TCGA/GEO cohorts; predominantly MIBC)	expression correlated with significantly shorter OS	estimated using MCP-Counter; WGCNA; IHC validation
(Du et al., 2022)	Myofibroblastic CAFs (myCAFs)	Bladder cancer across stages (NMIBC and MIBC; TCGA/GEO)	High myCAF gene signature was associated with poorer OS	myCAF gene signature (ssGSEA); MCP-Counter/xCell; IHC (ACTA2)
(Liu et al., 2023)	General CAFs (CAF gene signature)	Bladder urothelial carcinoma (training and validation cohorts; mainly MIBC)	High CAF-risk score independently predicted shorter OS	Integrated scRNA-seq and bulk RNA-seq; WGCNA; LASSO Cox model; CAF infiltration algorithms
(Zhang et al., 2023)	General CAF-related genes (CRGs)	Bladder cancer (TCGA and GEO; NMIBC and MIBC)	High CRG-risk score was associated with significantly worse OS	Transcriptomic analysis; unsupervised clustering; LASSO Cox regression
(Chen et al., 2022)	Inflammatory CAFs (iCAFs)	Bladder cancer (TCGA-BLCA, GEO meta-cohort; mainly MIBC)	iCAF-high subtype demonstrated significantly poorer OS	scRNA-seq–derived iCAF signature; consensus clustering; validation cohorts
(Muilwijk et al., 2021)	FAP-positive CAFs	High-grade T1 NMIBC	High stromal FAP expression predicted worse OS	IHC-based quantitative assessment of FAP expression
(Li et al., 2024)	General CAFs (fibroblast-related gene index)	Bladder cancer, predominantly MIBC (multi-cohort)	Fibroblast-high ("fibroblast-hot") tumors showed significantly worse OS	scRNA-seq and bulk RNA-seq integration; machine-learning–derived fibroblast gene index

Table 1 and **table 2** show that in analysis of TCGA-BLCA showed that patients with high CAF abundance had significantly worse overall survival (OS; $p = 0.003$). High CAF levels also correlated with more advanced clinicopathologic features – grade, stage, and T and N categories were all elevated in high-CAF tumors ($p < 0.001$ for each) (Du et al., 2021). Individual stromal markers were assessed by Cox regression and cluster analysis. High FAP expression predicted reduced OS (log-rank $p < 0.001$; HR=2.06, 95% CI 1.52–2.80; $p < 0.001$) and high PDGFRβ also conferred worse survival (HR=1.75, 95% CI 1.21–2.53; $p = 0.003$). After multiple-testing correction, only FAP and PDGFRβ remained significant. Cluster analysis showed that a FAP-dominant CAF cluster had the poorest 5-year OS (HR=2.25, 95% CI 1.08–4.67; $p = 0.030$) compared to an ASMA (αSMA) reference cluster (Mezheyeuski et al., 2020). BLCA patients were stratified by CAF gene expression into three clusters; OS differed significantly between clusters ($p = 0.002$). High CAF (myCAF) scores were associated with markedly shorter survival, high-CAF tumors showed reduced OS and disease-free survival (DFS) ($p < 0.001$ for both). In multivariate Cox models, high CAF score remained an independent risk factor (OS: $p = 0.049$; DFS: $p = 0.031$) (Du et al., 2022).

In another identified a seven-gene CAF signature (risk score) that strongly predicted survival. In TCGA-BLCA, each unit increase in CAF risk score raised the hazard of death (multivariate HR=3.513, 95% CI 1.997–6.182; $p < 0.001$). High-risk patients fared much worse: TCGA high- vs. low-risk groups had HR = 2.134 (95% CI 1.565–2.91; $p < 0.001$), and external validation in GSE cohorts likewise showed significant hazard ratios (e.g. HR = 2.927, 95% CI 1.26–6.801; $p = 0.009$) (Liu et al., 2023). Then on the approach to other research used five CAF-related genes (CRGs) to define two molecular subtypes. Patients in the CAF-low subtype had significantly better OS than those in the CAF-high subtype ($p < 0.001$). Consistently, high CRG-risk patients had markedly poorer survival (log-rank $p < 0.001$). Their prognostic model showed excellent discrimination (3-year OS AUC 0.88) (Zhang et al., 2023).

Then in BLCA which is classified based on 2 ICAF subtypes cluster 2 (high iCAF signature) was associated with significantly worse OS than Cluster 1: Kaplan–Meier analysis yielded $p = 0.024$ (Chen et al., 2022). Finally, a tissue-marker study found that stromal FAP protein strongly stratified outcome in T1 NMIBC. High FAP in tumor stroma predicted inferior recurrence-free, progression-free, cancer-specific, and overall survival compared to low FAP (log-rank $p \ll 0.05$ for each endpoint). FAP remained an independent prognostic factor after adjusting for BCG therapy (Muilwijk et al., 2021). Fibroblast gene index (FRGI) was created. A nomogram incorporating FRGI achieved very high accuracy (ICB-treated BLCA 1-year OS AUC=0.95). Notably, high FRGI scores were significantly enriched among checkpoint-inhibitor nonresponders ($p < 0.0001$) (Li et al., 2024).

Discussion

CAF Subtypes

Recent studies reveal a spectrum of CAF subtypes in bladder urothelial carcinoma, each with distinct

transcriptional profiles and biological properties. For example, predominant myofibroblastic CAF (myCAF) cluster characterized by smooth muscle and ECM-related gene expression (Wang et al., 2023). Distinguished an inflammatory CAF (iCAF) related subtype, their cluster 2 had high iCAF signature and was enriched for basal/squamous molecular features. CAF can defined fibroblast hot vs cold types based on a fibroblast gene index (FRGI), the hot type (high FRGI) showed higher immune/stromal scores and was predominantly basal-like (Li et al., 2024). These classifications align with emerging concepts that CAFs include contractile myCAFs and secretory/inflammatory iCAFs. the subtypes often cosegregate with patient features for instance, high-CAF subtypes correlate with higher stage and basal-differentiated tumors (Liu et al., 2022) . Clustering by CAF gene expression also stratifies survival, the low CAFs subtype had much better OS than the high CAFs subtype (Zhang et al., 2023) . Importantly, CAF subtypes interact with other TME components differently: in myCAF-high tumors (Wang et al., 2023), CD8 T cells and M2 macrophages were both elevated, whereas in iCAF-high tumors adaptive and innate immune signatures varied (Liu et al., 2022). The nomenclature and markers for CAF subtypes vary across studies, but a recurring theme is that one subtype tends to express contractile and ECM genes (often αSMA/myCAF) while another expresses inflammatory cytokines (iCAF). These data, from multiple cohorts and methodologies, underscore the plasticity of CAFs, a CAF subtype classification emerges that is tumor-promoting (CAF-hot/myCAF) or less (CAF-cold/low).

Biological Mechanisms of CAF

Several mechanistic studies explain how CAFs drive tumor progression and impact survival. CAFs remodel the extracellular matrix (ECM) and secrete factors that promote tumor cell growth and invasion. For instance, Gene Ontology analyses of CAF-associated genes highlight enrichment in ECM organization, focal adhesion, and cell–cell adhesion pathways. One CAF-associated gene, COL10A1, was expressed with many ECM-related genes and correlated with focal-adhesion signaling in BLCA (Wang et al., 2023). CAFs also participate in cross-talk loops they secrete cytokines that stimulate cancer cell proliferation and epithelial–mesenchymal transition, and they recruit immune cells to create a pro tumorigenic. CAFs in general encourage angiogenesis, metastasis, and ECM remodeling, and they can form an immunosuppressive feedback loop with other TME cells. Experimentally, conditioned medium from CAFs or CAF-derived exosomes has been shown to increase BLCA cell invasion and drug resistance (crosstalk via paracrine IL-6/STAT3). Moreover, CAF-secreted ECM components create a dense matrix that can hinder drug delivery and mechanically support tumor cell migration. The combined effect of these mechanisms is that CAF-rich tumors grow faster, invade earlier, and resist therapy, all contributing to worse OS (Liu et al., 2023).

CAF-Driven Immunosuppression and Resistance to Checkpoint Inhibitors

CAFs profoundly shape the immune microenvironment. They produce

immunosuppressive cytokines and attract regulatory immune cells. BLCA tumors with high myCAF scores had elevated levels of both $CD8^+$ T cells and immunosuppressive M2 macrophages. However, these $CD8^+$ T cells often become dysfunctional, high-CAF tumors showed upregulation of immune checkpoint and inflammatory gene signatures, yet TIDE analysis indicated severe T cell exclusion and dysfunction. In other words, CAFs appeared to physically sequester T cells in stroma and raise exhaustion markers. This translated clinically into resistance to immunotherapy tumors with high myCAF scores were significantly less responsive to PD-1/PD-L1 blockade (Du et al., 2022). Similarly, found that patients classified as fibroblast *hot* (high FRGI) were mainly those who failed ICB therapy. These findings are in line with known CAF functions CAFs can upregulate checkpoint ligands and secrete TGF-β to inactivate cytotoxic T cells, and they can recruit Tregs and MDSCs to dampen immunity (Li et al., 2024). Indeed, strong positive correlation between iCAF signature and markers of T cell exhaustion. Taken together, the data show CAFs create a pro tumoral immune attracting regulatory myeloid/T cells, excluding effector T cells, and rendering immunotherapy less effective (Wang et al., 2023).

Extracellular Matrix Remodeling

ECM modification by CAFs is another key pathway affecting survival. CAFs deposit collagen, fibronectin, and other ECM proteins that stiffen the stroma and facilitate invasion. High-CAF BLCA tumors exhibited upregulation of ECM remodeling pathways including focal adhesion and actin cytoskeleton regulation (Du et al., 2021). Likewise noted enrichment of collagen and adhesion signatures in the iCAF-high cluster. Experimental studies support that CAFs induce EMT and invasiveness in BLCA cells via matrix-related signals. Moreover, a dense ECM can physically block immune cells and drugs, amplifying therapeutic resistance. For example, multiple studies report that CAF-mediated ECM deposition contributes to chemoresistance in solid tumors. Thus, patients with CAF-rich tumors likely have more aggressive, invasion-prone disease with poorer drug penetration, a combination that shortens survival (Liu et al., 2022).

Risk Stratification and Treatment Selection

Given the above mechanisms, the prognostic CAF signatures have implications for risk stratification and therapy. High-CAF risk groups, identified by gene scores or protein markers (e.g. FAP or COL10A1), consistently map to worse OS and may indicate candidates for intensified therapy. Conversely, patients in CAF-low clusters have survival akin to lower-risk disease. This suggests CAF metrics could refine existing staging for example, a stage II patient with a high CAF score might behave like stage III. Moreover, CAF markers could guide treatment selection. CAF-derived effectively predicted response to cisplatin and to immunotherapy: nonresponders to ICB were concentrated in the high-CAF group13. Similarly, CAF signatures might identify tumors more likely to benefit from anti-CAF strategies (e.g. CAF-targeting antibodies or TGF-β inhibitors). The risk models achieve high

predictive accuracy (AUCs ~0.9 at 3–5 years), indicating clinical utility in prognostication. In sum, CAF-based classification adds a layer to risk models and might inform decisions on chemotherapy, immunotherapy, or novel CAF-targeted treatments (e.g. FAP inhibitors) (Liu et al., 2023 ; Zhang et al., 2023).

Prognostic Value

Evidence consistently indicates that CAF abundance and subtype composition bear strong prognostic significance in BLCA. Multiple studies report that higher CAF infiltration predicts poor outcomes. For example, high CAF scores in TCGA BLCA were significantly associated with reduced OS (Du et al., 2021), a finding echoed by risk-modeling studies where high CAF gene-risk groups had markedly worse survival (Liu et al., 2022 ; Zhang et al., 2023). Stromal markers like FAP also proved prognostic in T1 NMIBC, high FAP stromal index was strongly linked to shorter recurrence-free and overall survival. Conversely, patients in CAF-low subtypes exhibit better outcomes. CAF-low subtype patients survived significantly longer than CAF-high subtype patients. These findings suggest that CAF-level metrics (genes, proteins or computational scores) can stratify BLCA patients into high- vs low-risk groups. Importantly, the prognostic power of CAF signatures held up after multivariate adjustment in independent cohorts. Even after accounting for stage, age, or treatment, CAF markers remained significant, implying an effect beyond traditional clinical factors. This supports the view that CAFs are independent drivers of tumor aggressiveness. It should be noted that prognostic thresholds varied but the direction of effect was uniform: elevated CAF-related markers portend unfavorable prognosis. In summary, across diverse BLCA cohorts and analytical methods, CAF-related measures consistently emerged as adverse prognostic indicators (Zhang et al., 2023).

Managerial Implication

The evidence from recent bladder cancer studies supports moving CAF/stroma metrics from “research-only” into a standardized, operational layer of risk management. In particular, high stromal content (e.g., stroma–tumor ratio/STR) has been linked to worse prognosis and to a more immunosuppressive, PD-L1–enriched microenvironment, which means hospitals can use stromal assessment as a practical trigger for earlier multidisciplinary discussion, tighter follow-up planning, and more deliberate selection of systemic therapy strategies in patients flagged as “stroma-high.” (Da Y. et al. 2025).

Implementation-wise, feasibility is improving because stroma quantification no longer has to rely on subjective eyeballing. A machine-learning workflow applied to routine H&E whole-slide images has been shown to quantify TSR with prognostic value while reducing pathologist workload, which is directly relevant for management: it enables SOP-based reporting, QA/consistency monitoring, and scalability across sites (Zheng Q et al, 2023). In parallel, radiomics models derived from pelvic CT have been reported to non-invasively predict high TSR and to stratify likelihood of pathologic complete response to

neoadjuvant chemotherapy, suggesting an additional operational lever when tissue is limited or when pre-treatment triage is needed (e.g., prioritizing who should be fast-tracked to NAC pathways or clinical trials) (Liu et al. 2024). Finally, CAF-subtype risk scoring built from multi-cohort data has been associated with a non-inflamed phenotype and lower immunotherapy remission, supporting governance workflows where CAF-risk flags prompt earlier trial screening or combination-strategy consideration (e.g., stroma-modulating approaches alongside ICI) and better targeting of biomarker-testing resources to the patients most likely to benefit (Qin Y. et al. 2023).

Conclusion

Cancer-associated fibroblasts (CAFs) represent critical stromal components in bladder urothelial carcinoma (BLCA) progression and immunotherapy response. This systematic review of nine comprehensive studies demonstrates that CAF-related gene signatures and molecular subtypes provide robust prognostic stratification and predictive biomarkers for patient outcomes in bladder cancer.

The analyses identified distinct CAF subpopulations with divergent functional roles, inflammatory CAFs predominantly associated with immunosuppression and poor prognosis, myofibroblasts exhibiting bimodal effects on tumorigenesis and progression, and multiple other CAF phenotypes defined by specific markers. Multi-gene prognostic signatures incorporating CAF-related markers successfully predicted overall survival.

Key biomarkers and CAF signatures demonstrated independent prognostic value and significant correlations with immune infiltration and tumor mutational burden. These findings establish CAF characterization as a valuable approach for risk stratification and therapeutic decision-making in bladder cancer clinical practice.

From a managerial and implementation perspective, these findings support incorporating CAF-/stroma-oriented assessment (e.g., TSR/CAF-risk or CAF-subtype signatures) into routine bladder cancer workflows to strengthen risk stratification and guide therapy selection. Standardizing measurement through structured pathology reporting and scalable digital quantification can improve reproducibility across centers and enable earlier multidisciplinary escalation for CAF-/stroma-high cases. In practice, CAF-informed pathways may help allocate resources more efficiently by prioritizing intensive surveillance and clinical-trial/combination-strategy consideration for patients most likely to experience poor outcomes or reduced immunotherapy benefit.

Limitations & Future Directions

All the above findings must be viewed in light of limitations. Many analyses are based on retrospective cohorts and require prospective validation. The CAF signatures were derived and tested on the same data sources (TCGA, select GEO sets), raising the possibility of overfitting or cohort bias. Clinically, it remains to be shown that using CAF biomarkers changes patient outcomes. The studies also focus on bulk tissue or predefined markers; CAF heterogeneity at single-cell or

protein level is not fully captured. Thus, future work should include orthogonal validation of CAF classifiers – for example, comparing gene-expression–based CAF scores with histopathologic CAF quantification or single-cell fibroblast subtyping. In addition, functional studies are needed to confirm that targeting CAFs (e.g. by neutralizing CAF-secreted factors) will improve therapy response in BLCA. Finally, as new CAF subtypes are reported (e.g. antigen-presenting CAFs in other cancers), it will be important to map the full repertoire of CAF phenotypes in bladder cancer. Addressing these gaps will refine the prognostic models and pave the way to CAF-directed therapies in BLCA.

Acknowledgment

We would like to express our sincere gratitude to our partner institutions, Bhayangkara H.S. Samsoeri Mertojoso Hospital and Abdhi Family General Hospital, for their valuable support and collaboration throughout the activity. We also thank all hospital staff and contributing parties who assisted in coordinating and facilitating the implementation process. Their commitment, cooperation, and professionalism were essential to the smooth and successful completion of this activity. We greatly appreciate the time, effort, and dedication provided by everyone involved.

Conflict of Interest

The authors declare that there are no conflicts of interest related to the preparation and publication of this article.

Author Contribution

Vico Mardenanta: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Writing – Original Draft.

Jodii Arlan Kurnia: Methodology, Validation, Visualization, Writing – Review & Editing, Supervision, Project Administration.

References

Bellmunt, J., Hussain, M., Gschwend, J. E., Albers, P., Dann, J. M., De Santis, M., et al. (2021). Adjuvant atezolizumab versus observation in muscle-invasive urothelial carcinoma (IMvigor010): A multicentre, open-label, randomised, phase 3 trial. *The Lancet Oncology, 22*(4), 525–537.

Burley, A., Rullan, A., & Wilkins, A. (2022). A review of the biology and therapeutic implications of cancer-associated fibroblasts in muscle-invasive bladder cancer. *Frontiers in Oncology, 12*, 1000888.

Chen, H., Yang, W., Xue, X., Li, Y., Jin, Z., & Ji, Z. (2022). Integrated analysis revealed an inflammatory cancer-associated fibroblast-based subtypes with promising implications in predicting the prognosis and immunotherapeutic response of bladder cancer patients. *International Journal of Molecular Sciences, 23*(24), 15970.

Chen, H., Yang, W., Xue, X., Li, Y., Jin, Z., & Ji, Z. (2022). Integrated analysis revealed an inflammatory cancer-associated fibroblast-based subtypes with promising implications in predicting the prognosis and immunotherapeutic response of bladder cancer

patients. *International Journal of Molecular Sciences, 23*(24), 15970.

Da, Y., Lu, Z., Zhu, Z., Tai, H., Liu, Y., & Zhu, Y. (2025). A high stroma-tumor ratio is associated with an immunosuppressive tumor microenvironment and a poor prognosis in bladder cancer. *Frontiers in Oncology, 15*, 1604609. https://doi.org/10.3389/fonc.2025.1604609

Du, Y. H., Jiang, X., Wang, B., Cao, J., Wang, Y., Yu, J., Wang, X. Z., & Liu, H. T. (2021). The cancer-associated fibroblasts related gene CALD1 is a prognostic biomarker and correlated with immune infiltration in bladder cancer. *Cancer Cell International, 21*, 283.

Du, Y. H., Sui, Y. Q., Cao, J., Jiang, X., Wang, Y., Yu, J., Wang, B., Wang, X. Z., & Xue, B. X. (2022). Dynamic changes in myofibroblasts affect the carcinogenesis and prognosis of bladder cancer associated with tumor microenvironment remodeling. *Frontiers in Cell and Developmental Biology, 10*, 833578.

Koll, F. J., Banek, S., Kluth, L., Mende, C. H., Gontarewicz, A., Dräger, D. L., et al. (2023). Tumor-associated macrophages and Tregs influence and represent immune cell infiltration of muscle-invasive bladder cancer and predict prognosis. *Journal of Translational Medicine, 21*(1), 124.

Li, J. X., Kong, Z., Qi, Y. J., Wang, W., Su, Q., Huang, W., Zhang, Z. H., Li, S., & Du, E. (2024). Single-cell and bulk RNA-sequence identified fibroblasts signature and CD8+ T-cell-fibroblast subtype predicting prognosis and immune therapeutic response of bladder cancer. *International Journal of Surgery, 110*, 4911–4931.

Liu, J., Li, C., Huang, K., Zhang, L., Liu, B., Gao, S., et al. (2022). A classification based on tumor-stroma ratio and tumor budding for patients with muscle-invasive bladder cancer. *Expert Review of Anticancer Therapy, 22*(3), 323–330.

Liu, L., Xu, L., Wu, D., Zhu, Y., Li, X., Xu, C., Chen, K., Lin, Y., Lao, J., Cai, P., Li, X., Luo, Y., Li, X., Huang, J., Lin, T., & Zhong, W. (2024). Impact of tumour stroma-immune interactions on survival prognosis and response to neoadjuvant chemotherapy in bladder cancer. *eBioMedicine, 104*, 105152. https://doi.org/10.1016/j.ebiom.2024.105152

Liu, Y., Jian, J., Zhang, Y., Wang, L., Liu, X., & Chen, Z. (2023). Construction of cancer-associated fibroblasts related risk signature based on single-cell RNA-seq and bulk RNA-seq data in bladder urothelial carcinoma. *Frontiers in Oncology, 13*, 1170893.

Mezheyeuski, A., Segersten, U., Leiss, L. W., Malmström, P.-U., Hatina, J., Östman, A., et al. (2020). Fibroblasts in urothelial bladder cancer define stroma phenotypes that are associated with clinical outcome. *Scientific Reports, 10*(1), 281.

Muilwijk, T., Akand, M., Daelemans, S., Marien, K., Waumans, Y., Kockx, M., et al. (2021). Stromal marker fibroblast activation protein drives outcome in T1 non-muscle invasive bladder cancer. *PLOS ONE, 16*(9), e0257195.

Page, M. J., McKenzie, J. E., Bossuyt, P. M.,

Boutron, I., Hoffmann, T. C., Mulrow, C. D., *et al.* (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ, 372*, n71. https://doi.org/10.1136/bmj.n71

Qin, Y., Zu, X., Li, Y., Han, Y., Tan, J., Cai, C., Shen, E., Liu, P., Deng, G., Feng, Z., Wu, W., Peng, Y., Liu, Y., Ma, J., Zeng, S., Chen, Y., & Shen, H. (2023). A cancer-associated fibroblast subtypes-based signature enables the evaluation of immunotherapy response and prognosis in bladder cancer. *iScience, 26*(9), 107722. https://doi.org/10.1016/j.isci.2023.107722

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., & Jemal, A. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians, 71*(3), 209–249.

Wang, X., Bai, Y., Zhang, F., Li, D., Chen, K., Wu, R., Tang, Y., Wei, X., & Han, P. (2023). Prognostic value of COL10A1 and its correlation with tumor-infiltrating immune cells in urothelial bladder cancer: A comprehensive study based on bioinformatics and clinical analysis validation. *Frontiers in Immunology, 14*, 955949.

Wang, X., Bai, Y., Zhang, F., Li, D., Chen, K., Wu, R., Tang, Y., Wei, X., & Han, P. (2023). Prognostic value of COL10A1 and its correlation with tumor-infiltrating immune cells in urothelial bladder cancer: A comprehensive study based on bioinformatics and clinical analysis validation. *Frontiers in Immunology, 14*, 955949.

Zhang, M., Zhu, J., Zhang, P., Li, L., Min, M., Li, T., & He, W. (2023). Development and validation of cancer-associated fibroblasts-related gene landscape in prognosis and immune microenvironment of bladder cancer. *Frontiers in Oncology, 13*, 1174252.

Zheng, Q., Jiang, Z., Ni, X., Yang, S., Jiao, P., Wu, J., Xiong, L., Yuan, J., Wang, J., Jian, J., Wang, L., Yang, R., Chen, Z., & Liu, X. (2023). Machine learning quantified tumor-stroma ratio is an independent prognosticator in muscle-invasive bladder cancer. *International Journal of Molecular Sciences, 24*(3), 2746. https://doi.org/10.3390/ijms24032746