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尿路上皮癌免疫治疗生物标志物的研究进展、挑战与多维度整合策略

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摘　要

尿路上皮癌(UC)作为泌尿系统常见恶性肿瘤，免疫检查点抑制剂(ICIs)的临床应用显著改变了其治疗格局，但患者个体治疗反应差异巨大，精准预测疗效成为临床亟待解决的关键问题。生物标志物作为筛选获益人群、优化治疗策略的核心工具，近年来成为UC免疫治疗领域的研究热点。本文系统综述了经典生物标志物PD-L1、新兴标志物肿瘤突变负荷(TMB)及其他潜在标志物的研究现状，深入分析了当前生物标志物临床应用中存在的技术标准化不足、肿瘤异质性干扰、治疗背景影响等核心挑战，并探讨了多组学整合、联合治疗策略中的标志物应用等创新方向。最后，展望了未来通过技术标准化、机制深度解析及多维度评估体系构建，推动UC免疫治疗精准化发展的前景，为临床实践与科研探索提供参考。

关键词

尿路上皮癌，免疫治疗，生物标志物，PD-L1，肿瘤突变负荷，多组学整合

Research Progress, Challenges and Multidimensional Integration Strategies of Immunotherapy Biomarkers in Urothelial Carcinoma

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Abstract

Urothelial carcinoma (UC) is a common malignant tumor of the urinary system. The clinical application of immune checkpoint inhibitors (ICIs) has significantly changed the therapeutic landscape. However, the therapeutic responses vary greatly among individual patients, and precise prediction of efficacy has become an urgent clinical issue. As a core tool for selecting beneficiary populations and optimizing treatment strategies, biomarkers have emerged as a research hotspot in the field of immunotherapy for UC in recent years. This paper systematically reviews the research progress of classic biomarkers such as PD-L1, emerging biomarkers including tumor mutational burden (TMB), and other potential biomarkers. It deeply analyzes the key challenges in the clinical application of current biomarkers, including insufficient technical standardization, interference of tumor heterogeneity, and the influence of therapeutic context. Furthermore, it discusses innovative directions such as multi-omics integration and biomarker application in combination therapy. Finally, it prospects the future of promoting the precise development of UC immunotherapy through technical standardization, in-depth mechanistic analysis, and the establishment of a multidimensional evaluation system, so as to provide references for clinical practice and scientific research.

Keywords

Urothelial Carcinoma, Immunotherapy, Biomarkers, PD-L1, Tumor Mutational Burden, Multi-Omics Integration

1. 引言与研究价值定位

1.1. 尿路上皮癌的疾病负担与治疗困境

尿路上皮癌包括膀胱癌、上尿路尿路上皮癌等亚型，其发病率和死亡率在泌尿系统肿瘤中位居前列。传统治疗以手术、化疗、放疗为主，但晚期或转移性 UC 患者的治疗效果有限，5 年生存率较低[1]。化疗药物(如铂类联合方案)虽为晚期 UC 的一线治疗选择，但存在耐药性强、副作用明显等问题，多数患者在治疗后会出现疾病进展，需更高效、安全的治疗手段[2]。

1.2. 免疫治疗在尿路上皮癌中的突破性进展

免疫检查点抑制剂(ICIs)的问世为 UC 治疗带来了革命性突破。PD-1/PD-L1 抑制剂(如 atezolizumab、pembrolizumab 等)通过阻断免疫抑制信号，重新激活机体抗肿瘤免疫反应，已被批准用于晚期 UC 的一线或二线治疗[3]。多项临床试验证实，ICIs 可显著改善部分患者的客观缓解率和总生存期，为化疗耐药或不耐受的患者提供了新的治疗选择[4]。然而，ICIs 并非对所有 UC 患者有效，仅部分患者能从中长期获益[5]，这一现状凸显了精准筛选获益人群的重要性。

1.3. 生物标志物研究的临床必要性

UC 患者对 ICIs 的治疗反应存在显著个体差异，其根本原因在于肿瘤生物学特征、免疫微环境状态等存在异质性。可靠的生物标志物能够精准识别最可能从免疫治疗中获益的患者，避免无效治疗带来的

毒副作用和医疗资源浪费，同时为治疗方案的优化调整提供依据。现有研究表明，单一生物标志物难以全面覆盖 UC 的复杂异质性，整合组织与循环标志物、结合肿瘤微环境分析的多维度评估策略，可能成为突破当前治疗瓶颈的关键[6]。因此，系统解析 UC 免疫治疗生物标志物的作用机制、优化检测方法、构建整合评估体系，具有重要的临床价值与科研意义。

2. 经典生物标志物 PD-L1 的研究进展与争议

PD-L1 作为免疫检查点通路的核心分子，是 UC 免疫治疗中研究最早、最广泛的生物标志物，其表达水平与 ICIs 疗效的关联已得到大量临床数据支持，但同时也存在诸多争议与挑战。

2.1. PD-L1 表达检测的技术标准与判读争议

PD-L1 的检测主要依赖免疫组织化学(IHC)技术，但目前临床应用的检测试剂盒(如 Ventana SP142、SP263 和 Dako 22C3)在抗体特异性、染色靶点(肿瘤细胞/免疫细胞)及判读标准上存在显著差异，导致检测结果的一致性较差[7]。在 UC 中，SP142 试剂盒对免疫细胞的染色敏感性更高，而 22C3 和 SP263 主要聚焦于肿瘤细胞 PD-L1 表达水平的评估，这种检测靶点的差异直接影响患者免疫治疗适应症的判定[8][9]。Imvigor130 研究显示同一样本经 SP142 与 22C3 检测可分为双阳性、SP142 单阳性、22C3 单阳性、双阴性四种表型，不一致率高达 40% [10]。这种差异本质是微环境分型差异：SP142 识别“免疫活化型”，22C3 识别“肿瘤表达型”，二者无法直接互换，是导致疗效预测分歧的核心生物学原因。此外，PD-L1 表达具有动态性(治疗过程中可能发生表达变化)和空间异质性(原发灶与转移灶、肿瘤不同区域的表达水平存在差异)，进一步增加了临床判读的复杂性，亟需建立统一的技术流程、判读标准及质量控制体系[11][12]。

2.2. PD-L1 阳性率与免疫治疗响应的相关性

PD-L1 表达水平与 ICIs 疗效的关联是临床关注的核心问题，但现有研究结论尚未完全统一。多项研究显示，PD-L1 高表达患者(如 SP142 检测中免疫细胞浸润 ≥ 5%)对 PD-1/PD-L1 抑制剂的客观缓解率(ORR)和中位生存期(OS)显著优于低表达或阴性患者[13]。KEYNOTE-361 试验结果表明，PD-L1 阳性(CPS ≥ 10)的晚期 UC 患者接受 pembrolizumab 单药治疗的中位生存期明显长于化疗组，证实 PD-L1 高表达可作为 ICIs 治疗获益的预测因子[14]。然而，临床实践中也发现部分 PD-L1 阴性患者仍能从免疫治疗中获益，而部分 PD-L1 阳性患者却出现治疗无响应或快速进展，这一现象表明 PD-L1 作为单一生物标志物的预测局限性[15]。此外，IMvigor130 试验进一步证实，PD-L1 表达与 ICIs 疗效的相关性在不同检测方法间存在显著差异，同一患者可能因检测试剂盒选择不同而被划分为不同获益风险组，这一问题严重影响了 PD-L1 标志物的临床实用性[10]。

3. 新兴生物标志物的探索与验证

为弥补单一 PD-L1 标志物的不足，研究者们积极探索了其他具有预测价值的新兴生物标志物，其中肿瘤突变负荷(TMB)是最具潜力的候选标志物之一，同时其他基因改变及免疫微环境相关指标也展现出一定的应用前景。

3.1. 肿瘤突变负荷(TMB)的临床应用前景

TMB 指肿瘤基因组中每百万碱基对的突变数量，其通过增加肿瘤新抗原的产生，增强肿瘤的免疫原性，进而影响 ICIs 的治疗效果。多项研究已证实，TMB 是晚期 UC 中已确立的预测性生物标志物之一

[16]，高 TMB 患者接受 ICIs 治疗的响应率和生存期均显著优于低 TMB 患者。然而，TMB 在 UC 免疫治疗中的应用仍存在较多争议：首先，TMB 的阈值标准尚未统一，不同研究采用的检测平台、测序范围(全外显子测序/靶向测序)不同，导致界定高/低 TMB 的临界值差异较大，难以在临床中推广应用[17]；其次，特定基因改变可能影响 TMB 的预测价值，例如成纤维细胞生长因子受体 3 (FGFR3)突变的转移性 UC 患者，其 TMB 与 ICIs 疗效的关联较弱，需进一步阐明其作用机制[18]；此外，错配修复缺陷(MMR-deficient)和 DNA 聚合酶 ε (POLE)突变的 UC 患者通常表现出高 TMB 特征，且对 ICIs 治疗反应良好，这为亚组人群的精准筛选提供了依据[19]。另有病例报告描述了具有极高 TMB (伴随 PD-L1、PD-L2 和 PD-1 表达上调)的浸润性尿路上皮癌患者对免疫治疗的良好响应，为 TMB 高表达亚型的治疗策略优化提供了理论支持[20]。总体而言，TMB 作为 UC 免疫治疗预测标志物的临床应用前景广阔，但需解决阈值标准化、与其他标志物的协同作用等问题。

3.2. 其他潜在新兴生物标志物

除 TMB 外，多种基因改变和免疫微环境相关指标也被证实与 UC 免疫治疗疗效相关。例如，DNA 损伤修复(DDR)通路突变(如 BRCA1/2、ATM 突变)的 UC 患者，肿瘤免疫原性增强，可能对 ICIs 更敏感[21]；NKG2A/HLA-E 通路作为替代免疫检查点，其表达水平与 PD-L1 联合检测可提高疗效预测的准确性[22]。免疫微环境相关指标如肿瘤浸润淋巴细胞(TILs)的数量、亚型及功能状态也具有重要预测价值。三级淋巴结构(TLS)的存在与否、调节性 T 细胞(Treg)比例、细胞因子谱等，均可能作为辅助预测标志物，为 UC 免疫治疗的精准化提供更多维度的参考[23]。

4. 生物标志物临床应用的关键挑战

尽管 UC 免疫治疗生物标志物的研究取得了显著进展，但在临床转化过程中仍面临多重挑战，这些问题严重制约了其精准预测价值的发挥。

4.1. 现有标志物的预测准确性局限

单一生物标志物(如 PD-L1 或 TMB)的预测能力有限，无法准确覆盖所有响应人群[24] [25]，是当前最核心的挑战。PD-L1 表达作为经典生物标志物存在显著局限性。不同检测方法(如 Ventana SP142、SP263 和 Dako 22C3)的判读标准不一致，导致结果有差异，影响患者分层和治疗选择[26] [27]，TMB 缺乏统一阈值，且在特定基因背景下预测价值受限[28]。部分“生物标志物阴性”患者仍能从 ICIs 治疗中获益，而“阳性”患者可能出现耐药，这一现象表明现有标志物尚未完全捕获 UC 免疫治疗响应的复杂生物学机制[29]。此外，不同标志物之间的协同或拮抗作用尚不明确，缺乏有效的整合评估模型，进一步降低了临床预测的准确性。

4.2. BCG 免疫治疗相关症状对疗效评估的干扰

卡介苗(BCG)膀胱内灌注是肌层浸润性膀胱癌术前新辅助治疗或非肌层浸润性膀胱癌术后辅助治疗的常用方案，其对肿瘤微环境的重塑作用可能干扰后续 ICIs 治疗的疗效评估。研究表明，BCG 治疗可导致肿瘤浸润淋巴细胞(TILs)功能耗竭，降低其抗肿瘤活性——从 BCG 治疗后原发肿瘤中扩增的 TILs，其抗肿瘤活性显著低于未接受 BCG 治疗的患者(74.3% vs.未治疗组) [30]。此外，肿瘤病理亚型也会影响这种干扰效应，混合型尿路上皮癌的 TILs 反应性显著高于纯尿路上皮癌(89.5% vs. 56.3%) [30]。BCG 治疗引发的免疫微环境异质性，使得基于 TILs 评分、PD-L1 表达等生物标志物的预测模型难以准确评估 ICIs 疗效，给临床决策带来困难。

5. 多组学整合的创新研究方向

面对 UC 的高度异质性和单一生物标志物的局限性，多组学整合策略通过整合基因组学、转录组学、蛋白质组学、免疫微环境分析等多维度数据，全面解析肿瘤生物学特征与免疫治疗响应的关联，成为推动精准治疗的核心方向。

5.1. 基因组学与免疫微环境的交叉分析

基因组学改变(如基因突变、拷贝数变异、表观遗传修饰)与免疫微环境重塑密切相关，二者的交叉分析为揭示 UC 免疫治疗响应机制提供了重要视角。大规模多组学研究证实，驱动基因突变可通过调控信号通路影响肿瘤免疫微环境状态：例如，HRAS 突变通过调控 mTOR 信号通路，促进乳头状尿路上皮瘤而非浸润性癌的形成，同时影响免疫细胞浸润模式[31]；DNA 甲基化等表观遗传改变可调控免疫相关基因的表达，进而塑造免疫抑制或活化的肿瘤微环境[32] [33]。通过全外显子测序、RNA 测序等技术，整合驱动基因突变谱与免疫微环境特征(如 TILs 亚型、细胞因子表达)，可识别出与 ICIs 疗效相关的分子亚型，为精准筛选获益人群提供依据。此外，对原发灶和转移灶的多组学比对分析，能够揭示肿瘤异质性的分子基础，为转移灶生物标志物的替代检测提供参考。

5.2. 多组学整合案例[31]

案例：基于基因组 + 转录组 + 蛋白质组 + 磷酸化蛋白质组的多组学整合解析 UC 进展与免疫异质性

研究设计：作者对 190 例 UC 患者的 448 份 FFPE 样本开展全外显子测序、转录组、蛋白质组、磷酸化蛋白质组整合分析，覆盖正常、增生、乳头状瘤、乳头状尿路上皮癌(PUC)、原位癌(CIS)、浸润癌等全病程阶段，构建 UC 进展多组学图谱。

应用与效果：明确 UC 两条独立进展分支：PUC 型以 HRAS 突变、糖脂代谢增强、免疫浸润降低为特征；CIS 型以 TP53 突变、DNA 损伤修复(DDR)激活、APOBEC 突变特征富集、免疫浸润高为特征。建立可区分浸润癌来源的 18 蛋白分类器，将浸润癌分为 PUC 来源与 CIS 来源，CIS 来源型预后更差、转移风险更高，与免疫治疗响应直接相关。发现 8p12 缺失及靶基因 RBPMS 缺失通过激活 AP‑1 转录因子促进转移，RBPMS 可作为 UC 转移抑制标志物与治疗靶点。

遇到的问题：FFPE 样本多组学检测稳定性不足；多组学数据维度高、计算复杂，临床难以快速落地；单点活检难以反映时空异质性。

启示：多组学整合可实现分子分型、预后分层、靶点发现三位一体，突破单一标志物局限；CIS/PUC 分型可直接指导免疫治疗策略选择。

6. 联合治疗策略中的生物标志物价值

免疫治疗联合其他治疗手段(如抗血管生成治疗、化疗、放疗)已成为 UC 治疗的重要发展趋势，生物标志物在联合治疗方案的选择、疗效评估及治疗顺序优化中发挥着关键作用。

6.1. 免疫－抗血管生成联合治疗的协同机制与标志物应用

免疫检查点抑制剂与抗血管生成药物的联合应用，通过协同重塑肿瘤微环境实现增效作用。抗血管生成药物(如 VEGF 抑制剂)可抑制肿瘤血管异常增生，改善肿瘤微环境缺氧状态，同时降低调节性 T 细胞(Treg)活性、增加细胞毒性 T 淋巴细胞浸润，从而增强 ICIs 的抗肿瘤效应[34]。在这一联合策略中，生物标志物可用于筛选最可能获益的患者：例如，VEGF 表达水平、肿瘤血管密度可作为预测联合治疗响

应的潜在指标；PD-L1 高表达且 VEGF 阳性的 UC 患者，可能从该联合方案中获得更显著的生存获益[35]。

6.2. 化疗/放疗与免疫治疗联合的标志物指导价值

化疗与放疗可通过诱导肿瘤细胞免疫原性死亡、释放肿瘤新抗原、增强抗原提呈能力，为免疫治疗创造有利条件，二者的联合应用已在 UC 治疗中展现出良好前景。铂类化疗后，肿瘤微环境中 CD8+T 细胞浸润增加，PD-L1 表达水平动态上调，这一变化可作为序贯免疫治疗的时机选择依据[36]；放疗可激活干扰素信号通路、增强主要组织相容性复合体(MHC)分子表达，但其疗效依赖于肿瘤的免疫激活状态，TMB 水平、T 细胞受体多样性等标志物可用于评估放疗后免疫激活程度，指导后续免疫治疗的联合应用[37]。生物标志物还可用于优化联合治疗顺序：例如，对于 PD-L1 高表达、TMB 高的患者，可优先采用免疫单药治疗；而对于 PD-L1 低表达、免疫微环境抑制明显的患者，先给予化疗或放疗重塑微环境，再序贯免疫治疗可能获得更优疗效[38]。

6.3. 基于生物标志物的治疗顺序优化

联合治疗的疗效不仅依赖于治疗手段的选择，还与治疗顺序密切相关，生物标志物为治疗顺序的个体化优化提供了依据。对于 PD-L1 高表达或免疫激活型 UC 患者(如 CD8+ T 细胞浸润丰富、TMB 高)，免疫单药治疗或免疫联合抗血管生成治疗可作为首选方案；对于 PD-L1 低表达但存在 DDR 通路突变的患者，化疗联合免疫治疗可能更适合；而对于 BCG 治疗后复发、TILs 功能耗竭的患者，优先采用免疫检查点抑制剂联合免疫调节剂(如 IL-2)的方案，可能更有效地逆转免疫抑制状态[39]。此外，动态监测生物标志物的表达变化(如治疗过程中 PD-L1 表达、TMB 水平的动态调整)，可及时评估治疗响应，为联合治疗方案的实时优化提供支持。

7. 未来展望与结论

当前 PD-L1 检测存在显著的技术标准差异，不同检测方法(如 SP142 与 22C3)在尿路上皮癌中呈现不一致的结果，导致判读争议和临床预测价值受限[40]。例如，IMvigor130 试验中两种检测方法对同一样本的 PD-L1 表达评估差异高达 40%，凸显标准化体系的急迫性[41] [42]。此外，肿瘤异质性(如原发灶与转移灶的 PD-L1 表达差异)进一步削弱单一检测的可靠性。因此，还需建立统一的检测流程、判读标准及质控体系，以提升生物标志物临床应用的准确性与可重复性[43]。

PD-1/PD-L1 轴的调控需超越单纯表达水平检测，转向多维度机制探索。研究表明，干扰素-γ (IFN-γ)通过 JNK 信号通路调控 PD-L1 mRNA 的 m6A 甲基化修饰，影响其稳定性[44]；RNF144A 介导 PD-L1 的泛素化降解，而 FGFR3 激活可抑制此过程，导致 PD-L1 异常积累[45] [46]。此外，表观遗传调控(如 DNA 甲基化)和免疫微环境因子(如 NKG2A)与 PD-L1 协同作用，可优化免疫治疗响应预测[47] [48]。

未来技术路径可以整合：

动态监测技术：如单细胞测序解析 PD-1/PD-L1 信号在 T 细胞耗竭中的时空异质性[49]；

联合靶向策略：如 PI3K 抑制剂与 PD-1/PD-L1 阻断剂的协同机制探索[50]；

人工智能模型：通过多组学数据建模预测 PD-1/PD-L1 通路活性[51]。

单一生物标志物(如 PD-L1 或 TMB)的预测准确性不足(仅 20%~30%患者响应免疫治疗)，需转向多组学整合的系统评估[52] [53]。关键方向包括：多组学交叉分析：基因组学(如 DDR 通路突变)、免疫微环境特征(如 TILs 评分、三级淋巴结构)及代谢组学的联合分析可揭示响应机制[54]-[57]。例如，免疫激活型肿瘤中 CD8+T 细胞浸润与 PD-L1 空间分布的关联性。

参考文献

[1] Siegel, R.L., Miller, K.D., Wagle, N.S. and Jemal, A. (2023) Cancer Statistics, 2023. *CA*: *A Cancer Journal for Clinicians*, **73**, 17-48. https://doi.org/10.3322/caac.21763

[2] Vuky, J., Balar, A.V., Castellano, D., O'Donnell, P.H., Grivas, P., Bellmunt, J., *et al*. (2020) Long-Term Outcomes in KEYNOTE-052: Phase II Study Investigating First-Line Pembrolizumab in Cisplatin-Ineligible Patients with Locally Advanced or Metastatic Urothelial Cancer. *Journal of Clinical Oncology*, **38**, 2658-2666. https://doi.org/10.1200/jco.19.01213

[3] Rosenberg, J.E., Hoffman-Censits, J., Powles, T., van der Heijden, M.S., Balar, A.V., Necchi, A., *et al*. (2016) Atezolizumab in Patients with Locally Advanced and Metastatic Urothelial Carcinoma Who Have Progressed Following Treatment with Platinum-Based Chemotherapy: A Single-Arm, Multicentre, Phase 2 Trial. *The Lancet*, **387**, 1909-1920. https://doi.org/10.1016/s0140-6736(16)00561-4

[4] Bellmunt, J., de Wit, R., Vaughn, D.J., Fradet, Y., Lee, J., Fong, L., *et al*. (2017) Pembrolizumab as Second-Line Therapy for Advanced Urothelial Carcinoma. *New England Journal of Medicine*, **376**, 1015-1026. https://doi.org/10.1056/nejmoa1613683

[5] Xie, Q., Zheng, H., Chen, Y. and Peng, X. (2022) Corrigendum: Cost-Effectiveness of Avelumab Maintenance Therapy Plus Best Supportive Care vs. Best Supportive Care Alone for Advanced or Metastatic Urothelial Carcinoma. *Frontiers in Public Health*, **10**, Article ID: 965798. https://doi.org/10.3389/fpubh.2022.965798

[6] Rani, B., Ignatz-Hoover, J.J., Rana, P.S. and Driscoll, J.J. (2023) Current and Emerging Strategies to Treat Urothelial Carcinoma. *Cancers*, **15**, Article 4886. https://doi.org/10.3390/cancers15194886

[7] Rimm, D.L., Han, G., Taube, J.M., Yi, E.S., Bridge, J.A., Flieder, D.B., *et al*. (2017) A Prospective, Multi-Institutional, Pathologist-Based Assessment of 4 Immunohistochemistry Assays for PD-L1 Expression in Non-Small Cell Lung Cancer. *JAMA Oncology*, **3**, 1051-1058. https://doi.org/10.1001/jamaoncol.2017.0013

[8] Munari, E., Querzoli, G., Brunelli, M., Marconi, M., Sommaggio, M., Cocchi, M.A., *et al*. (2022) Comparison of Three Validated PD-L1 Immunohistochemical Assays in Urothelial Carcinoma of the Bladder: Interchangeability and Issues Related to Patient Selection. *Frontiers in Immunology*, **13**, Article ID: 954910. https://doi.org/10.3389/fimmu.2022.954910

[9] Karunamurthy, A. and Davar, D. (2025) There and Back Again: PD-L1 Positivity as a Biomarker for Immune Checkpoint Blockade in Urothelial Carcinoma. *Cancer Immunology Research*, **13**, 454-455. https://doi.org/10.1158/2326-6066.cir-25-0202

[10] Galsky, M.D., Kockx, M., Roels, J., Van Elzen, R., Guan, X., Yuen, K., *et al*. (2025) Different PD-L1 Assays Reveal Distinct Immunobiology and Clinical Outcomes in Urothelial Cancer. *Cancer Immunology Research*, **13**, 476-486. https://doi.org/10.1158/2326-6066.cir-24-0649

[11] Erlmeier, F., Klümper, N., Landgraf, L., Strissel, P.L., Strick, R., Sikic, D., *et al*. (2023) Spatial Immunephenotypes of Distant Metastases but Not Matched Primary Urothelial Carcinomas Predict Response to Immune Checkpoint Inhibition. *European Urology*, **83**, 133-142. https://doi.org/10.1016/j.eururo.2022.10.020

[12] de Andrea, C.E., Abengozar-Muela, M., Arranz, J.Á., Climent, M.A., Puente, J., Vizcay, Á., *et al*. (2025) Association of PD-1, LAG-3 and TIM-3 Expression on Intratumoral CD8 T-Cells with Response to Atezolizumab in a Real-World-Evidence Biomarker Study for Advanced Urothelial Carcinoma Patients. *OncoImmunology*, **14**, Article 2538687. https://doi.org/10.1080/2162402x.2025.2538687

[13] Maiorano, B.A., Di Maio, M., Cerbone, L., Maiello, E., Procopio, G., Roviello, G., *et al*. (2024) Significance of PD-L1 in Metastatic Urothelial Carcinoma Treated with Immune Checkpoint Inhibitors: A Systematic Review and Meta-Analysis. *JAMA Network Open*, **7**, e241215. https://doi.org/10.1001/jamanetworkopen.2024.1215

[14] Powles, T., Csőszi, T., Özgüroğlu, M., Matsubara, N., Géczi, L., Cheng, S.Y., *et al*. (2021) Pembrolizumab Alone or Combined with Chemotherapy versus Chemotherapy as First-Line Therapy for Advanced Urothelial Carcinoma (KEYNOTE-361): A Randomised, Open-Label, Phase 3 Trial. *The Lancet Oncology*, **22**, 931-945. https://doi.org/10.1016/s1470-2045(21)00152-2

[15] Szabados, B., Prendergast, A., Jackson-Spence, F., Choy, J. and Powles, T. (2021) Immune Checkpoint Inhibitors in Front-Line Therapy for Urothelial Cancer. *European Urology Oncology*, **4**, 943-947. https://doi.org/10.1016/j.euo.2021.02.010

[16] De Souza, A.L., Mega, A.E., Douglass, J., Olszewski, A.J., Gamsiz Uzun, E.D., Uzun, A., Chou, C., Duan, F., Wang, J., *et al*. (2023) Clinical Features of Patients with MTAP-Deleted Bladder Cancer. *American Journal of Cancer Research*, **13**, 326-339.

[17] Wang, Z., Huang, D., Li, S. and Ke, L. (2025) Role of Tumor Mutational Burden in Patients with Urothelial Carcinoma Treated with Immune Checkpoint Inhibitors: A Systematic Review and Meta-Analysis. *Frontiers in Immunology*, **16**, Article ID: 1592761. https://doi.org/10.3389/fimmu.2025.1592761

[18] Gupta, S., Lee, J.K., Mitchell, J. and Huang, R.S.P. (2025) Fibroblast Growth Factor Receptor 3 Alteration Status and Outcomes on Immune Checkpoint Inhibitors in Patients with Metastatic Urothelial Carcinoma. *JCO Precision Oncology*, **9**, e2500257. https://doi.org/10.1200/po-25-00257

[19] Hwang, H.S., Kim, D. and Choi, J. (2021) Distinct Mutational Profile and Immune Microenvironment in Microsatellite-Unstable and *Pole*-Mutated Tumors. *Journal for ImmunoTherapy of Cancer*, **9**, e002797. https://doi.org/10.1136/jitc-2021-002797

[20] Scimeca, M., Bischof, J., Bonfiglio, R., Nale, E., Iacovelli, V., Carilli, M., *et al*. (2024) Molecular Profiling of a Bladder Cancer with Very High Tumour Mutational Burden. *Cell Death Discovery*, **10**, Article No. 202. https://doi.org/10.1038/s41420-024-01883-x

[21] Felsenstein, K.M. and Theodorescu, D. (2018) Precision Medicine for Urothelial Bladder Cancer: Update on Tumour Genomics and Immunotherapy. *Nature Reviews Urology*, **15**, 92-111. https://doi.org/10.1038/nrurol.2017.179

[22] Cai, J., Song, L., Zhang, F., Wu, S., Zhu, G., Zhang, P., *et al*. (2024) Targeting SRSF10 Might Inhibit M2 Macrophage Polarization and Potentiate Anti-PD-1 Therapy in Hepatocellular Carcinoma. *Cancer Communications*, **44**, 1231-1260. https://doi.org/10.1002/cac2.12607

[23] Sautès-Fridman, C., Petitprez, F., Calderaro, J. and Fridman, W.H. (2019) Tertiary Lymphoid Structures in the Era of Cancer Immunotherapy. *Nature Reviews Cancer*, **19**, 307-325. https://doi.org/10.1038/s41568-019-0144-6

[24] Klümper, N., Wüst, L., Saal, J., Ralser, D.J., Zarbl, R., Jarczyk, J., *et al*. (2023) PD-L1 (CD274) Promoter Hypomethylation Predicts Immunotherapy Response in Metastatic Urothelial Carcinoma. *OncoImmunology*, **12**, Article 2267744. https://doi.org/10.1080/2162402x.2023.2267744

[25] Dijk, N.V., Gil-Jimenez, A., Silina, K., Montfoort, M.L.v., Einerhand, S., Jonkman, L., *et al*. (2021) The Tumor Immune Landscape and Architecture of Tertiary Lymphoid Structures in Urothelial Cancer. *Frontiers in Immunology*, **12**, Article ID: 793964. https://doi.org/10.3389/fimmu.2021.793964

[26] Koti, M., Bivalacqua, T., Black, P.C., Cathomen, T., Galsky, M.D., Gulley, J.L., *et al*. (2023) Adaptive Immunity in Genitourinary Cancers. *European Urology Oncology*, **6**, 263-272. https://doi.org/10.1016/j.euo.2023.03.002

[27] Jensen, H.A.R., Horsbøl, T.A., Thygesen, L.C., Davidsen, M., Christensen, A.I. and Ekholm, O. (2023) Variations in the Agreement of Self-Reported Cancer: A Danish Nationwide Study. *International Journal of Cancer*, **154**, 217-225. https://doi.org/10.1002/ijc.34692

[28] Jin, X., Wang, Q., Luo, F., Pan, J., Lu, T., Zhao, Y., *et al*. (2023) Single-Cell Transcriptomic Analysis of Tumor Heterogeneity and Intercellular Networks in Human Urothelial Carcinoma. *Chinese Medical Journal*, **136**, 690-706. https://doi.org/10.1097/cm9.0000000000002573

[29] Jasinski-Bergner, S., Eckstein, M., Taubert, H., Wach, S., Fiebig, C., Strick, R., *et al*. (2022) The Human Leukocyte Antigen G as an Immune Escape Mechanism and Novel Therapeutic Target in Urological Tumors. *Frontiers in Immunology*, **13**, Article ID: 811200. https://doi.org/10.3389/fimmu.2022.811200

[30] Aydin, A.M., Bunch, B.L., Beatty, M., Hajiran, A., Dhillon, J., Sarnaik, A.A., *et al*. (2021) The Factors Affecting Expansion of Reactive Tumor Infiltrating Lymphocytes (TIL) from Bladder Cancer and Potential Therapeutic Applications. *Frontiers in Immunology*, **12**, Article ID: 628063. https://doi.org/10.3389/fimmu.2021.628063

[31] Yao, Z., Xu, N., Shang, G., Wang, H., Tao, H., Wang, Y., *et al*. (2023) Proteogenomics of Different Urothelial Bladder Cancer Stages Reveals Distinct Molecular Features for Papillary Cancer and Carcinoma in Situ. *Nature Communications*, **14**, Article No. 5670. https://doi.org/10.1038/s41467-023-41139-3

[32] Zhu, C., Wang, Y., Zhu, R., Wang, S., Xue, J., Zhang, D., *et al*. (2024) Gut Microbiota and Metabolites Signatures of Clinical Response in Anti-PD-1/PD-L1 Based Immunotherapy of Biliary Tract Cancer. *Biomarker Research*, **12**, Article No. 56. https://doi.org/10.1186/s40364-024-00607-8

[33] Peng, M. (2022) Immune Landscape of Distinct Subtypes in Urothelial Carcinoma Based on Immune Gene Profile. *Frontiers in Immunology*, **13**, Article ID: 970885. https://doi.org/10.3389/fimmu.2022.970885

[34] Liu, Z., Zang, M., Li, K., Qi, W., Yuan, H., Chen, L., *et al*. (2024) The Immunotherapy-Based Combination Associated Score as a Robust Predictor for Outcome and Response to Combination of Immunotherapy and VEGF Inhibitors in Renal Cell Carcinoma. *Computers in Biology and Medicine*, **182**, Article 109210. https://doi.org/10.1016/j.compbiomed.2024.109210

[35] He, X., Liu, X., Zuo, F., Shi, H. and Jing, J. (2023) Artificial Intelligence-Based Multi-Omics Analysis Fuels Cancer Precision Medicine. *Seminars in Cancer Biology*, **88**, 187-200. https://doi.org/10.1016/j.semcancer.2022.12.009

[36] Michaud, É., Mansure, J.J. and Kassouf, W. (2024) Integrating Novel Immunotherapeutic Approaches in Organ-preserving Therapies for Bladder Cancer. *British Journal of Pharmacology*, **183**, 1215-1232. https://doi.org/10.1111/bph.16300

[37] Meric-Bernstam, F., Larkin, J., Tabernero, J. and Bonini, C. (2021) Enhancing Anti-Tumour Efficacy with Immunotherapy Combinations. *The Lancet*, **397**, 1010-1022. https://doi.org/10.1016/s0140-6736(20)32598-8

[38] Tsuji, S., Reil, K., Nelson, K., Proclivo, V.H., McGuire, K.L. and Giacalone, M.J. (2022) Intravesical VAX014 Synergizes with PD-L1 Blockade to Enhance Local and Systemic Control of Bladder Cancer. *Cancer Immunology Research*, **10**, 978-995. https://doi.org/10.1158/2326-6066.cir-21-0879

[39] Balar, A.V., Castellano, D., O'Donnell, P.H., Grivas, P., Vuky, J., Powles, T., *et al*. (2017) First-Line Pembrolizumab in Cisplatin-Ineligible Patients with Locally Advanced and Unresectable or Metastatic Urothelial Cancer (KEYNOTE-052): A Multicentre, Single-Arm, Phase 2 Study. *The Lancet Oncology*, **18**, 1483-1492. https://doi.org/10.1016/s1470-2045(17)30616-2

[40] Sathyanarayanan, A., Gupta, R., Thompson, E.W., Nyholt, D.R., Bauer, D.C. and Nagaraj, S.H. (2019) A Comparative Study of Multi-Omics Integration Tools for Cancer Driver Gene Identification and Tumour Subtyping. *Briefings in Bioinformatics*, **21**, 1920-1936. https://doi.org/10.1093/bib/bbz121

[41] Wu, C., Duan, L., Li, H., Liu, X., Cai, T., Yang, Y., *et al*. (2023) $Pd1^{hi}$ $Cd200^{hi}$ $Cd4^{+}$ Exhausted T Cell Increase Immunotherapy Resistance and Tumour Progression by Promoting Epithelial-Mesenchymal Transition in Bladder Cancer. *Clinical and Translational Medicine*, **13**, e1303. https://doi.org/10.1002/ctm2.1303

[42] Ren, Y., Ren, F. and Yang, B. (2025) Multi-Layer Matrix Factorization for Cancer Subtyping Using Full and Partial Multi-Omics Dataset. *Briefings in Bioinformatics*, **26**, bbaf448. https://doi.org/10.1093/bib/bbaf448

[43] Lee, S., Choi, H.Y., Lee, G., Kim, T., Cho, H., Oh, I., *et al*. (2021) $Cd8^{+}$ Tils in NSCLC Differentiate into TEMRA via a Bifurcated Trajectory: Deciphering Immunogenicity of Tumor Antigens. *Journal for ImmunoTherapy of Cancer*, **9**, e002709. https://doi.org/10.1136/jitc-2021-002709

[44] Ni, Z., Sun, P., Zheng, J., Wu, M., Yang, C., Cheng, M., *et al*. (2022) JNK Signaling Promotes Bladder Cancer Immune Escape by Regulating Mettl3-Mediated M6a Modification of PD-L1 mRNA. *Cancer Research*, **82**, 1789-1802. https://doi.org/10.1158/0008-5472.can-21-1323

[45] Jing, W., Wang, G., Cui, Z., Xiong, G., Jiang, X., Li, Y., *et al*. (2022) FGFR3 Destabilizes PD-L1 via NEDD4 to Control T-Cell-Mediated Bladder Cancer Immune Surveillance. *Cancer Research*, **82**, 114-129. https://doi.org/10.1158/0008-5472.can-21-2362

[46] Ho, S., Lee, Y., Ittmann, M.M., Lin, F., Chan, K.S. and Lin, W. (2021) RNF144A Deficiency Promotes PD-L1 Protein Stabilization and Carcinogen-Induced Bladder Tumorigenesis. *Cancer Letters*, **520**, 344-360. https://doi.org/10.1016/j.canlet.2021.08.008

[47] Yan, S., Zeng, H., Jin, K., Shao, F., Liu, Z., Chang, Y., *et al*. (2022) NKG2A and PD-L1 Expression Panel Predicts Clinical Benefits from Adjuvant Chemotherapy and PD-L1 Blockade in Muscle-Invasive Bladder Cancer. *Journal for ImmunoTherapy of Cancer*, **10**, e004569. https://doi.org/10.1136/jitc-2022-004569

[48] Salomé, B., Sfakianos, J.P., Ranti, D., Daza, J., Bieber, C., Charap, A., *et al*. (2022) NKG2A and HLA-E Define an Alternative Immune Checkpoint Axis in Bladder Cancer. *Cancer Cell*, **40**, 1027-1043.e9. https://doi.org/10.1016/j.ccell.2022.08.005

[49] Abdel-Hafiz, H.A., Schafer, J.M., Chen, X., Xiao, T., Gauntner, T.D., Li, Z., *et al*. (2024) Author Correction: Y Chromosome Loss in Cancer Drives Growth by Evasion of Adaptive Immunity. *Nature*, **626**, E11-E11. https://doi.org/10.1038/s41586-024-07104-w

[50] Zhu, S., Ma, A., Zhu, Z., Adib, E., Rao, T., Li, N., *et al*. (2021) Synergistic Antitumor Activity of Pan-Pi3k Inhibition and Immune Checkpoint Blockade in Bladder Cancer. *Journal for ImmunoTherapy of Cancer*, **9**, e002917. https://doi.org/10.1136/jitc-2021-002917

[51] Oisakede, E.O., Akinro, O., Bello, O.J., Analikwu, C.C., Egbon, E. and Olawade, D.B. (2025) Predictive Models for Immune Checkpoint Inhibitor Response in Cancer: A Review of Current Approaches and Future Directions. *Critical Reviews in Oncology/Hematology*, **216**, Article 104980. https://doi.org/10.1016/j.critrevonc.2025.104980

[52] Child, F., Lunj, S., Gough, J., Humphries, M.J., Biolatti, L.V., Hoskin, P.J., *et al*. (2025) The Hypoxic ECM and Neutrophils in MIBC Immunotherapy Resistance. *Nature Reviews Urology*, **23**, 236-255. https://doi.org/10.1038/s41585-025-01092-w

[53] Guo, Y., Dai, Y., Yin, J., Song, Y., *et al*. (2024) Novel Tumor Gene Expression Signatures Improve the Overall Survival Prediction Efficiency over Tumor Mutation Burden and PD-L1 Expression in Bladder Carcinoma with Checkpoint Blockade Immunotherapy. *American Journal of Cancer Research*, **14**, 4411-4428. https://doi.org/10.62347/timd7591

[54] Boll, L.M., Vázquez Montes de Oca, S., Camarena, M.E., Castelo, R., Bellmunt, J., Perera-Bel, J., *et al*. (2025) Predicting Immunotherapy Response of Advanced Bladder Cancer through a Meta-Analysis of Six Independent Cohorts. *Nature Communications*, **16**, Article No. 1213. https://doi.org/10.1038/s41467-025-56462-0

[55] Irwin, M.S. and Goldsmith, K.C. (2024) Current and Emerging Biomarkers: Impact on Risk Stratification for Neuroblastoma. *Journal of the National Comprehensive Cancer Network*, **22**, e247051. https://doi.org/10.6004/jnccn.2024.7051

[56] Yang, C., Ou, Y., Zhou, Q., Liang, Y., Li, W., Chen, Y., *et al*. (2023) Methionine Orchestrates the Metabolism Vulnerability in Cisplatin Resistant Bladder Cancer Microenvironment. *Cell Death & Disease*, **14**, Article No. 525.

https://doi.org/10.1038/s41419-023-06050-1

[57] Goubet, A., Lordello, L., Alves Costa Silva, C., Peguillet, I., Gazzano, M., Mbogning-Fonkou, M.D., *et al*. (2022) *Escherichia Coli*-Specific Cxcl13-Producing TFH Are Associated with Clinical Efficacy of Neoadjuvant PD-1 Blockade against Muscle-Invasive Bladder Cancer. *Cancer Discovery*, **12**, 2280-2307. https://doi.org/10.1158/2159-8290.cd-22-0201