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Challenges in the modelling of the pharmacokinetics and pharmacodynamics of an anti-cancer drug combination on urinary bladder cancer T24 cells: a technical review

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ABSTRACT

The development of novel anticancer therapies requires rigorous preclinical evaluation in order to ensure both efficacy and safety prior to clinical translation. The T24 cell line, derived from human transitional bladder carcinoma, is a widely employed *in vitro* model owing to its aggressive phenotype and strong relevance to human disease pathology. It offers a valuable platform for investigating bladder cancer progression, drug responsiveness, and mechanisms of resistance. However, replicating the complex tumour microenvironment and accurately predicting *in vivo* therapeutic outcomes still pose significant challenges *in vitro*. The pharmacokinetics (PK) and pharmacodynamics (PD) of candidate agents must be carefully assessed, as these parameters often diverge markedly between *in vitro* and *in vivo* contexts. *In vitro* PK/PD modelling – linking drug concentration to biological effect – facilitates dose optimization, particularly when integrated with pulsatile drug administration and advanced methodologies that simulate the three-dimensional tumour microenvironment. Such approaches enhance the translational relevance of bladder cancer research and may improve therapeutic outcomes. This review underscores the critical importance of optimizing the experimental design of *in vitro* PK/PD models in an attempt to address prevailing limitations and advance bladder cancer therapy.

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1. Introduction

Bladder cancer is among the most prevalent urological malignancies, marked by high recurrence rates and considerable morbidity, particularly in advanced stages. The T24 bladder cancer cell line, derived from a high-grade transitional cell carcinoma, is a widely used *in vitro* model for investigating tumour biology, mechanisms of drug resistance, and therapeutic responses[1]. Owing to its aggressive phenotype, it serves as a valuable platform for evaluating the efficacy of novel anticancer agents, including chemotherapeutics, targeted therapies, and combination regimens[2]. However, preclinical assessments using the T24 cell line present distinct challenges, especially in the integration of pharmacokinetics (PK) and pharmacodynamics (PD), both of which are essential for predicting clinical outcomes[1].

In vitro PK/PD modelling seeks to establish a quantitative relationship between drug concentration and biological effect, thereby supporting dose optimization and refinement of therapeutic strategies. Nonetheless, the application of conventional *in vitro* models is inherently limited. A major constraint is the inability of traditional two-dimensional (2D) monolayer cultures to replicate the structural and functional complexity of the three-dimensional (3D) tumour microenvironment (TME), which critically influences drug penetration, cell–cell interactions, and resistance mechanisms[3]. Moreover, standard *in vitro* systems lack dynamic physiological conditions such as blood flow, immune surveillance, and metabolic clearance, thereby impeding the translation of preclinical findings to clinical settings.

In an attempt to enhance the predictive value of *in vitro* PK/PD studies involving the T24 cell line, researchers must adopt innovative approaches that address these limitations[1]. Optimizing drug exposure conditions – such as pulsatile rather than continuous administration – alongside refined dose–response analyses and combination therapy evaluations, can yield a more comprehensive understanding of drug action and resistance. By closely mimicking the *in vivo* tumour environment through advanced experimental methodologies, *in vitro* PK/PD modelling can accelerate the development of more effective bladder cancer therapies, ultimately improving patient survival and quality of life[4]. This review underscores the importance of optimizing the experimental design of *in vitro* PK/PD models in order to overcome prevailing therapeutic challenges.

2. Replicating the TME *in vitro*

The TME plays a central role in cancer progression, therapeutic resistance, and drug responsiveness. It comprises a dynamic and heterogeneous network of cellular and extracellular components, including fibroblasts, immune cells, endothelial cells, and matrix proteins. Conventional 2D cell cultures fail to replicate the structural and functional intricacies of the TME. To address these limitations, 3D cell culture models have emerged as superior *in vitro* systems that more accurately simulate the *in vivo* tumour context. For example, co-culturing T24 bladder cancer cells with fibroblasts enables detailed investigation of stromal-mediated drug resistance, particularly through monitoring transforming growth factor-beta (TGF-β) and hepatocyte growth factor signalling pathways[5].

In vitro PK/PD modelling remains a cornerstone of preclinical drug development, aiming to quantify the temporal relationship between drug concentration and biological effect. *In vivo*, drug behaviour is modulated by physiological factors such as perfusion, tissue permeability, enzymatic metabolism, and clearance; features that are difficult to replicate under static *in vitro* conditions. Nevertheless, *in vitro* PK/PD systems have been engineered to simulate these pharmacokinetic processes, allowing for controlled assessment of drug exposure and efficacy[6].

The integration of 3D culture platforms with PK/PD modelling offers a more physiologically relevant framework for evaluating drug combinations and optimizing treatment protocols. By refining both 3D culture techniques and *in vitro* PK/PD methodologies, researchers can bridge the translational gap between preclinical testing and clinical application, thereby advancing personalized therapeutic strategies for bladder cancer[7] (Table 1).

3. Interpretation of *in vitro* PK/PD data regarding drug combination

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Table 1. Key studies that (directly or indirectly) inform our understanding of the challenges and advances in the *in vitro* pharmacokinetics / pharmacodynamics (PK/PD) modelling for bladder cancer using the T24 cell line.

Aspect	Details	Reference
Preclinical evaluation for anticancer therapies	Using the T24 cell line (derived from human transitional bladder carcinoma); strong relevance to human disease pathology; predicting *in vivo* therapeutic responses	Pinto-Leite *et al.* (2009)[1]
Limitations of conventional 2D models	Employing a 3D tumor microenvironment, leading to good predicting of *in vitro* drug responses (cell–cell interactions)	Barbosa *et al.* (2021)[2]
In vitro PK/PD model development	*In vitro* PK/PD model development achieves high accuracy of drug effects	Jihad *et al.* (2024)[4]
Cancer-associated fibroblasts in bladder cancer	The use of fibroblasts with the T24 cell line provides deeper insights for the study of drug resistance	Caramelo *et al.* (2023)[5]
In vitro PK/PD modelling	The employed *in vitro* PK/PD modelling sheds light on the relationship between drug concentration and biological effects over time, thereby aiding dose optimization	Abdalwahd *et al.* (2024)[6]
Bridge between *in vitro* and *in vivo* models by 3D cell cultures	Integration of a 3D culture with an *in vitro* PK/PD model gives good interpretation between *in vitro* and clinical data	Urzì *et al.* (2023)[7]
Intra-tumoral heterogeneity of bladder cancer	Intra- and inter-tumoral heterogeneity might complicate the evaluation of anti-cancer therapies	Warrick *et al.* (2019)[8]
Quantitative systems to predict *in vivo* efficacy from *in vitro* data	*In vitro* PK/PD model drug levels fluctuate with time, leading to well estimation of drug efficacy (simulating time-dependent drug exposure)	Bouhaddou *et al.* (2020)[9]
Mechanisms and challenges of cancer drug resistance	Drug resistance is a major obstacle in cancer treatment, often arising from genetic mutations, epigenetic changes, or adaptive responses	Khan *et al.* (2024)[10]

Bladder cancer exhibits pronounced intra- and inter-tumoral heterogeneity, complicating the evaluation of anticancer therapies. Although the T24 cell line is a valuable model, it represents only a single subtype of bladder cancer and may not fully capture the disease's biological diversity. Interpreting *in vitro* PK/PD data in the context of *in vivo* and clinical settings remains a significant challenge[8]. Conventional *in vitro* models often employ static drug concentrations, whereas both *in vivo* and advanced *in vitro* PK/PD systems simulate fluctuating drug levels over time; an approach that more accurately reflects clinical pharmacodynamics and improves the estimation of therapeutic efficacy.

By simulating time-dependent drug exposure in T24 cells, one can yield more reliable predictions of treatment outcomes, particularly for combination chemotherapy regimens[9]. Furthermore, drug resistance remains a major barrier to effective cancer therapy, frequently arising from genetic mutations, epigenetic alterations, or adaptive responses within the TME. Long-term exposure of T24 cells to sub-lethal drug concentrations can induce resistance, thereby enabling the study of underlying mechanisms and the evaluation of strategies to overcome them[10] (Table 1).

4. Conclusion

The evaluation of novel anticancer combinations using *in vitro* PK/PD modelling in the T24 bladder cancer cell line holds considerable promise, yet is accompanied by methodological and translational challenges. The standardization of these models, their integration into drug development pipelines, and the validation

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of their clinical relevance are essential next steps. By addressing these issues, researchers can enhance the interpretability of *in vitro* PK/PD data in relation to *in vivo* and clinical studies, thereby accelerating the development of effective bladder cancer therapies.

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Conflicts of interest

None exist.

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