Anticancer Prodrugs Targeting the Tumor Microenvironment and Their Clinical Advances

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Abstract. Targeting the tumor microenvironment (TME) has become a critical strategy in the development of anticancer prodrugs. This article reviews advances in the research of anticancer prodrugs utilizing TME-targeting strategies, including Aldoxorubicin, NC-6300, OBI-3424, and Legubicin. Aldoxorubicin employs a pH-sensitive hydrazone bond to conjugate doxorubicin to albumin, designed to release the active drug in the acidic TME. In a Phase III clinical trial for soft tissue sarcoma, it significantly prolonged progression-free survival (PFS) compared to standard chemotherapy; however, it increased hematological toxicity and gastrointestinal adverse events, without significantly reducing cardiotoxicity. NC-6300 enhances systemic stability through its micellar carrier; its maximum tolerated dose (MTD) far exceeds that of conventional epirubicin, and it demonstrates significantly improved cardiac safety at high cumulative doses, yet its objective response rate (ORR) is remarkably low (only 5%). OBI-3424 and Legubicin target TME-overexpressed enzymes AKR1C3 and Legumain, respectively, for activation. OBI-3424 showed significant efficacy in preclinical models of T-cell leukemia with high AKR1C3 expression; in clinical studies for solid tumors, it exhibited manageable safety but low ORR (2.6%), with efficacy highly dependent on tumor AKR1C3 expression levels. Legubicin utilizes a Legumain-cleavable peptide linker to release doxorubicin; preclinical studies indicated it significantly increases drug exposure at tumor sites while drastically reducing cardiac and renal toxicity, leading to an improved MTD. Prodrug strategies targeting the TME can enhance safety or enable targeted killing. However, the key challenge lies in balancing prodrug stability with targeted release capability. Optimizing this balance represents a crucial future direction for TME-targeted therapeutics.

Keywords: TME-targeting, Anticancer, Prodrugs.

1 Introduction

The tumor microenvironment, a multidimensional and dynamic system of interactions between cancer cells and the host, has emerged as a pivotal focus in cancer research. Stromal cells, collagen, signaling molecules, and unique physicochemical characteristics within the TME collectively drive malignant progression and therapeutic resistance

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[1]. Among these, acidosis represents one of the most distinctive features: due to the Warburg effect, cancer cells lower tumor tissue pH to 6.5–6.9 (vs. normal tissue pH 7.4) via glycolysis. This shift not only promotes tumor invasion and metastasis but also activates lysosomal cathepsins and matrix metalloproteinases to degrade the extracellular matrix [1]. Abnormal enzymatic activity in the TME similarly offers critical targeting opportunities; for instance, aldo-keto reductase AKR1C3 is upregulated 6–8-fold in liver and prostate cancers, while Legumain enzyme is specifically activated in tumor-associated macrophages, driving hormone-metabolism resistance and protease-mediated invasion/metastasis, respectively [2, 3]. In recent years, prodrug systems designed around TME characteristics have rapidly advanced, with their core logic leveraging microenvironment specificity to achieve targeted drug release—aiming to maximize local tumor drug concentration while minimizing normal tissue toxicity. However, the spatiotemporal heterogeneity of the TME poses significant challenges for precise prodrug delivery: uneven acid gradients, fluctuating enzyme expression levels, and immune-cell-mediated drug clearance mechanisms represent key optimization areas for future development. Understanding and manipulating the multidimensional features of the TME will determine the clinical translation efficacy of next-generation targeted chemotherapy.

2 Aldoxorubicin

2.1 Introduction to Aldoxorubicin

Aldoxorubicin is a novel targeted chemotherapeutic agent formed by conjugating doxorubicin to a maleimide group via an acid-labile hydrazone bond [4] (Figure 1). The maleimide moiety of this construct covalently binds to cysteine 34 of circulating albumin, forming an inactive prodrug [5]. This linker remains stable under normal physiological conditions but rapidly cleaves in the acidic tumor microenvironment or lysosomes, releasing free doxorubicin [6]. This targeted release mechanism seeks to minimize exposure of normal tissues—particularly cardiomyocytes—to active doxorubicin. Pharmacokinetic studies demonstrate that Aldoxorubicin circulates predominantly in its stable form, exhibiting a prolonged half-life compared to conventional doxorubicin. Enhanced drug accumulation in tumor tissues occurs alongside minimal systemic free doxorubicin concentration due to linker stability, thereby improving therapeutic safety without compromising antitumor efficacy [7]. The design of this drug overcomes the dose limitations traditionally associated with anthracyclines, enabling higher doses to be tested for treating solid tumors like soft tissue sarcoma and glioblastoma, with its long-term efficacy and safety currently under clinical investigation.

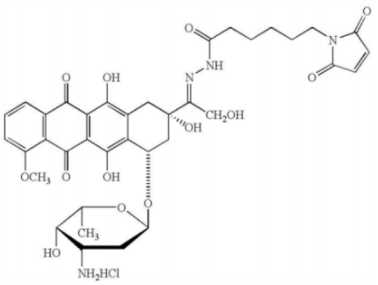

Fig. 1. Chemical structure of aldoxorubicin.

2.2 Phase III Clinical Trial of Aldoxorubicin in Soft Tissue Sarcoma

A multicenter, randomized, open-label Phase III study evaluated Aldoxorubicin versus investigator's choice (IC) of therapy in metastatic, locally advanced, or unresectable soft tissue sarcoma patients, comparing PFS and safety. The trial enrolled 433 patients, with 218 assigned to the Aldoxorubicin group and 215 to the IC group. Aldoxorubicin was administered at 350 mg/m² (equivalent to 260 mg/m² of doxorubicin), while the IC group received standard chemotherapy regimens, including doxorubicin. Results showed a significantly improved median PFS (mPFS) of 4.04 months in the Aldoxorubicin group compared to 2.96 months in the IC group. However, all-cause adverse events were higher with Aldoxorubicin (69.48% vs. 63.29%), and serious adverse events occurred more frequently (42.72% vs. 32.85%). Febrile neutropenia was markedly elevated in the Aldoxorubicin group (14.08% vs. 2.90% in IC). Notably, cardiotoxicity rates were unexpectedly higher with Aldoxorubicin (1.88% vs. 0.96%), contradicting its design rationale. Hematologic toxicities were consistently worse: neutropenia (29.58% vs. 18.84%), febrile neutropenia (15.02% vs. 4.35%), leukopenia (8.92% vs. 4.83%), and anemia (45.07% vs. 29.95%). Gastrointestinal adverse events were also more prevalent with Aldoxorubicin, including nausea (50.70% vs. 43.96%), stomatitis (53.52% vs. 14.98%), vomiting (28.17% vs. 21.26%), and constipation (29.58% vs. 19.54%), though diarrhea was slightly reduced (22.54% vs. 29.95%). In summary, while Aldoxorubicin demonstrated a significant PFS advantage over standard chemotherapy, it exhibited heightened systemic toxicity without mitigating cardiotoxicity.

3 NC-6300

3.1 Introduction to NC-6300

NC-6300 is a novel epirubicin prodrug in which epirubicin is covalently conjugated to the amphiphilic block copolymer PEG-PAsp (polyethylene glycol-polyaspartic acid) via an acid-cleavable hydrazone bond [8]. This conjugate self-assembles in aqueous solution to form nanosized micelles (40–80 nm diameter) with a hydrophilic PEG shell surrounding a hydrophobic drug core. Similar to Aldoxorubicin, NC-6300 employs the

pH-sensitive hydrazone linker, enabling selective epirubicin release in the acidic tumor microenvironment [9]. The micellar structure significantly enhances physicochemical stability in systemic circulation, protecting the drug from premature release, reducing off-target toxicity, and improving overall safety [10]. Phase I clinical trials demonstrated its advantages over conventional epirubicin in treating advanced or recurrent solid tumors, including a significantly higher cumulative dose tolerance and markedly reduced cardiotoxicity [8, 11].

3.2 Phase I Clinical Trial of NC-6300

An open-label, non-randomized Phase I dose-escalation study evaluated NC-6300 in 19 patients with advanced solid tumors refractory to standard therapy or lacking treatment options. Primary endpoints included MTD and dose-limiting toxicities (DLTs), while secondary endpoints encompassed pharmacokinetics (PK), ORR, and disease control rate (DCR) to assess safety, tolerability, and preliminary efficacy [8]. Dose escalation employed a traditional 3+3 design (15, 30, 60 mg/m²) in Phase I and a Bayesian continual reassessment model (CRM) in Phase II (80, 100, 130, 170, 225 mg/m²). Patients received 10-minute intravenous infusions every three weeks until disease progression or unacceptable toxicity. DLTs were defined as ≥grade 3 non-hematologic toxicity, ≥grade 4 hematologic toxicity, or febrile neutropenia. The MTD was established at 170 mg/m²—significantly higher than conventional epirubicin—with DLTs observed in 2 of 7 patients (29%) at this dose. Common adverse events included neutropenia (74%, grade 3–4 in 53%), leukopenia (74%), and anemia (42%). Serious adverse events were rare, with only one drug-related case of retinal hemorrhage. Notably, cardiotoxicity was substantially reduced: all patients maintained left ventricular ejection fraction (LVEF) ≥50%; 4 patients received cumulative doses >900 mg/m² (maximum 2,250 mg/m²), far exceeding epirubicin's limit; and no cardiotoxicity occurred even after >1 year of treatment. Efficacy analysis showed a low ORR (5%, one partial response in breast cancer at 100 mg/m²) but a high DCR of 57% and median progression-free survival (mPFS) ranging from 78 to 561 days. NC-6300 demonstrated improved safety over epirubicin and modest antitumor activity in advanced solid tumors.

4 AKR1C3-Targeted Prodrug: OBI-3424

4.1 Introduction to AKR1C3 and OBI-3424

Aldo-keto reductase 1C3 (AKR1C3), a member of the aldo-keto reductase (AKR) superfamily, was initially cloned from human prostate and placental cDNA libraries [12, 13]. AKR1C3 is closely implicated in cancer cell proliferation and is overexpressed across diverse malignancies [2, 14]. Capitalizing on this tumor-specific upregulation, OBI-3424 was developed as an AKR1C3-targeted anticancer prodrug. OBI-3424 is a chemically synthesized potent nitrogen mustard prodrug that undergoes selective cleavage by AKR1C3 in the presence of NADPH to release the bis-alkylating agent bisaziridine [15] (Figure 2). This activated compound induces interstrand and intrastrand DNA crosslinks, thereby inducing cell death during proliferation and exerting potent

antitumor effects [15]. This targeted activation mechanism aims to enhance tumor-selective cytotoxicity while minimizing toxicity to healthy tissues. Currently in clinical development for T-cell acute lymphoblastic leukemia (T-ALL) and advanced solid tumors, preclinical studies demonstrate OBI-3424's profound efficacy in T-ALL models, highlighting its potential as a promising therapeutic approach for aggressive, chemotherapy-resistant T-ALL [16].

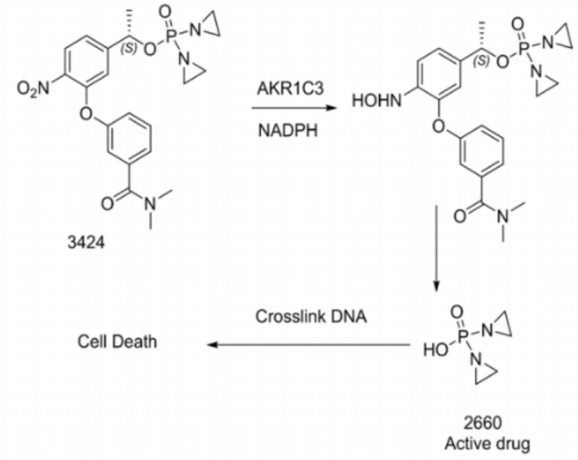

Fig. 2. Chemical atructure and activation process of OBI-3424.

4.2 Preclinical Study of OBI-3424 in T-cell Acute Lymphoblastic Leukemia (T-ALL)

AKR1C3 expression across pediatric ALL subtypes was evaluated, with RNA sequencing of patient-derived xenografts (PDXs) revealing significantly higher AKR1C3 mRNA levels in primary T-ALL compared to B-ALL, indicating greater therapeutic potential for OBI-3424 in T-ALL. In vitro cytotoxicity assays demonstrated potent activity against T-ALL cell lines, with an IC₅₀ of 9.7 nM—significantly lower than against B-ALL (60.3 nM) or early T-precursor ALL (ETP-ALL, 31.5 nM). Pre-treatment with the AKR1C3 inhibitor TH-3021 substantially attenuated OBI-3424's activity, confirming AKR1C3-dependent activation. In vivo efficacy assessments using pediatric ALL PDX models showed that OBI-3424 monotherapy (2.5 mg/kg weekly ×3 weeks) significantly extended event-free survival (EFS) by 17.1–77.8 days across 9 T-ALL models. Tumor regression was observed in 8 of 9 models, including 2 complete responses (CR) and 6 cases of minimal residual disease (MRD).

4.3 Clinical Study of OBI-3424

This study assessed the safety, PK, and clinical activity of OBI-3424 in patients with advanced or metastatic solid tumors [17]. Primary endpoints included incidence of dose-limiting toxicities (DLT), MTD, recommended Phase II dose (RP2D), and PK

characteristics, while also evaluating efficacy and safety. Through dose-escalation trials, the RP2D was established at 12 mg/m² administered every three weeks, demonstrating a manageable safety profile. Treatment-emergent adverse events (TEAEs) occurred in 32 of 39 patients (82%), most commonly anemia (64%), thrombocytopenia (49%), nausea (26%), and fatigue (21%). Three patients experienced serious adverse events (SAEs), with no drug-related deaths. Pharmacokinetic analysis revealed OBI-3424 has a short half-life (0.21–0.74 hours) and dose-dependent exposure. Exposure to the activated metabolite bis-aziridine—as measured by AUC—was substantially lower (1.4%–3.4% of OBI-3424 levels), though with a longer half-life (1.87–3.08 hours). Preliminary efficacy assessment showed one partial response (PR) in a cholangiocarcinoma patient (33% tumor reduction). Disease stabilization (SD) was observed in 21 patients (64%), with 15 maintaining SD for ≥12 weeks. Notably, the PR patient exhibited exceptionally high AKR1C3 expression (IHC h-score 205), suggesting enhanced clinical benefit in tumors with elevated AKR1C3.

5 Legumain-Targeted Prodrug: Legubicin

5.1 Introduction to Legumain and Legubicin

Legumain (asparaginyl endopeptidase or δ-secretase), a lysosomal cysteine protease with stringent substrate specificity, cleaves peptide bonds C-terminal to asparagine residues at approximately pH 5.8 [17, 18]. Overexpression of legumain occurs across multiple solid tumors—including breast, ovarian, prostate, and colon cancers, melanoma, and CNS tumors—and is particularly prominent in tumor-associated macrophages [3, 19]. This tumor-selective overexpression and unique proteolytic activity position legumain as a compelling therapeutic target, inspiring innovative drug designs such as Legubicin. Legubicin is a novel small-molecule conjugate comprising three elements: a 6-maleimidocaproyl (EMC) group, a tetrapeptide linker (Ala-Ala-Asn-Leu), and doxorubicin [20] (Figure 3). The tetrapeptide linker covalently connects the EMC moiety to the cytotoxic payload (doxorubicin) to form an inactive prodrug. Functionally analogous to its role in Aldoxorubicin, the EMC group forms a stable thioether bond with Cys34 of plasma albumin through Michael addition [20]. This albumin-drug complex shields doxorubicin within albumin's structure, reducing free-state cytotoxicity and extending systemic half-life. Upon reaching tumor tissue, highly expressed legumain cleaves the peptide bond between Asn and Leu in the tetrapeptide linker, releasing the doxorubicin precursor Leu-DOX [21]. Tissue peptidases subsequently convert Leu-DOX to active doxorubicin, enabling tumor-localized cytotoxicity [21]. This mechanism confers potent tumor-specific drug activation while substantially mitigating systemic toxicity, particularly cardiotoxicity, thus addressing the critical limitations of native doxorubicin.

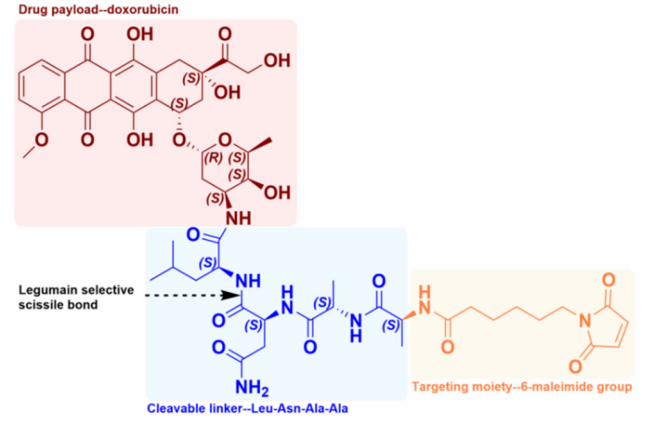

Fig. 3. Chemical structure of legubicin, comprising the EMC moiety, tetrapeptide linker (Ala-Ala-Asn-Leu), and doxorubicin

5.2 Preclinical Study of Legubicin

In tumor-bearing mouse models, comparative biodistribution analysis of Legubicin versus doxorubicin (administered intravenously at equimolar doses) revealed distinct drug exposure profiles [22]. Legubicin circulated predominantly in albumin-bound form, with minimal free doxorubicin in plasma. Tumor tissue exhibited a 1.77-fold increase in active doxorubicin exposure (AUC) with Legubicin versus conventional doxorubicin. Conversely, Legubicin significantly reduced exposure in normal tissues: cardiac AUC decreased by 3.26-fold, renal by 3.46-fold, and plasma by 1.29-fold. Acute toxicity studies in rats and dogs demonstrated substantially improved safety margins: MTD in rats reached 16 mg/kg (doxorubicin-equivalent) for females and 11 mg/kg for males (vs. 10.51 mg/kg for doxorubicin), while in dogs, MTD was 8 mg/kg (vs. 1.5 mg/kg for doxorubicin) [22]. Cardiotoxicity was markedly attenuated, with only mild cardiomyocyte degeneration observed at MTD. Toxicokinetic analysis confirmed minimal free doxorubicin and intermediate metabolite Leu-Dox in kidneys, heart, and plasma—consistent with albumin-mediated protection. Recent clinical development includes a Phase I dose-escalation trial initiated in 2020, followed by a multicenter randomized double-blind Phase II/III study (2021) evaluating Legubicin versus active comparator in advanced soft tissue sarcoma. As of 2025, an ongoing Phase I trial continues to assess safety, tolerability, pharmacokinetics, and preliminary efficacy in advanced solid tumors. Legubicin demonstrates significant potential as a next-generation tumor-targeted prodrug.

6 Conclusion

Prodrug designs targeting the TME achieve toxicity decoupling by enhancing systemic stability, yet balancing safety and efficacy hinges critically on prodrug dynamics in circulation versus the lesion microenvironment. The analyses above reveal an inverse

correlation between prodrug stability and toxicity: Aldoxorubicin exacerbated hematological toxicity due to premature hydrazone bond cleavage, while NC-6300's micellar plasma stability overcame epirubicin's dose limits and significantly reduced cardiotoxicity. However, enhanced stability often compromises efficacy; NC-6300's mere 5% ORR stems from insufficient micellar drug release at tumor sites, highlighting how excessive stability dampens target-site activation. For enzyme-activated prodrugs (e.g., AKR1C3/Legumain-targeted OBI-3424/Legubicin), efficacy confronts a distinct paradox: activation relies directly on enzymatic activity levels, but current detection methods poorly reflect catalytic efficiency. OBI-3424 achieved partial responses only in patients with high AKR1C3 expression (IHC h-score ≥200), failing in low-expression cohorts. Legubicin, though boosting tumor drug accumulation by 1.77 times, remains constrained by spatial heterogeneity in Legumain activity. This implies prodrug dissociation efficiency depends not only on enzyme abundance but also crucially on microenvironmental variables (local pH, cofactor concentration) and enzymatic kinetics—parameters often overestimated in preclinical models. Thus, optimizing prodrug design requires establishing dynamic enzyme activity monitoring and tailoring linker cleavage thresholds to microenvironmental parameters, ultimately reconciling the stability-activability dichotomy.

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