# Reframing cancer drug resistance: transporters, persister cells, senescence, and emerging therapeutic strategies

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*Cancer drug resistance remains one of the greatest barriers to durable therapeutic success. Here, we reframe resistance as a dynamic landscape shaped by transporter-mediated efflux, reversible drug-tolerant persister states, and therapy-induced senescence. This review highlights areas where our group has made substantial contributions, with a particular focus on our own experimental and conceptual advances. We show how P-glycoprotein (P-gp) overexpression, traditionally viewed as an obstacle sustaining cancer multidrug resistance (MDR), creates exploitable metabolic vulnerabilities, enabling the development of MDR-selective compounds. We demonstrate that persister cells rely on transient P-gp–mediated detoxification, revealing a therapeutic window during drug-free intervals. We further show that senescence is not a terminal fate but a relapse-initiating state requiring targeted intervention. Finally, we illustrate how pharmacokinetics and dosing schedules can be harnessed to reshape tumor evolution, culminating in LiPyDau, a next-generation liposomal anthracycline with durable activity against resistant tumors. Together, these insights outline a unified strategy for anticipating, intercepting, and ultimately overcoming cancer drug resistance. Magy Onkol 70:118-127, 2026*

***Keywords:*** *cancer, drug resistance, cellular senescence, persister cells, therapy protocols, LiPyDau*

A daganatellenes kezelésekkel szembeni rezisztencia továbbra is az egyik legjelentősebb akadálya a tartós terápiás sikernek. Összefoglalónk a rezisztenciát dinamikus rendszerként értelmezi újra, melyet az effluxtranszporterek, reverzibilis, gyógyszertoleráns perzisztер állapotok, valamint a terápiaindukált szeneszcencia együttesen formálnak. Rámutatunk, hogy a P-glikoprotein túlzott expressziója – hagyományosan a daganatok multidrog-rezisztenciájának fenntartója – kihasználható metabolikus sérülékenységeket hoz létre, miközben a perzisztер sejtek átmenetileg a P-gp-mediált méregtelenítésre támaszkodnak, ami terápiás ablakot nyit a gyógyszermentes időszakokban. Azt is bemutatjuk, hogy a szeneszcencia nem végállapot, hanem a relapszus kiindulópontja, amely célzott beavatkozást igényel. Végül szemléltetjük, miként formálhatók a tumorevolúciót meghatározó folyamatok farmakokinetikai jellemzők és adagolási protokollok segítségével, amelyekre építve megszületett a LiPyDau, egy új generációs liposzomális antraciklin, tartós hatékonysággal rezisztens daganatok ellen. E felismerések együtt egységes stratégiát körvonalaznak a gyógyszer-rezisztencia előrejelzésére, kivédésére és végső soron leküzdésére.

Nagy P, Kis AK, Bajtai E, Haffaressas IL, Gombos B, Bereczki D, Szebényi K, Szakács G, Tóth S, Füredi A. A daganatok gyógyszer-rezisztenciájának újrakeretezése: transzporterek, perzisztер sejtek, szeneszcencia és új terápiás stratégiák. Magy Onkol 70:118-127, 2026

**Kulcsszavak:** rák, gyógyszer-rezisztencia, sejtes szeneszcencia, perzisztер sejtek, terápiás protokollok, LiPyDau

## INTRODUCTION

Cancer remains one of the most formidable global health challenges, responsible for millions of deaths annually despite remarkable advances in early detection, molecular diagnostics, targeted therapies, and immuno-oncology (1). A central reason for this persistent burden is the ability of malignant cells to evade the cytotoxic effects of anticancer agents. Drug resistance, whether intrinsic or acquired, continues to undermine the long-term success of nearly every systemic treatment modality (2). Among the diverse mechanisms that enable tumor cells to survive chemotherapy, multidrug resistance (MDR) stands out as one of the most clinically significant and biologically complex (3).

Multidrug resistance refers to the phenomenon whereby cancer cells become simultaneously resistant to multiple structurally and mechanistically distinct chemotherapeutic agents. This broad resistance phenotype is frequently mediated by the overexpression of ATP-binding cassette (ABC) transporters, which actively efflux cytotoxic compounds from the intracellular space. Among these transporters, P-glycoprotein (P-gp, ABCB1) is the most extensively studied and arguably the most influential in shaping clinical resistance patterns. P-gp's ability to transport a wide range of hydrophobic chemotherapeutics, including anthracyclines, taxanes, vinca alkaloids, and epipodophyllotoxins, makes it a central determinant of treatment failure in several malignancies (4), as high P-gp expression marks tumors with reduced sensitivity to standard therapies and increased risk of early relapse across multiple tumor types (5–7), thus serving as a clinically relevant indicator of chemoresistance.

Beyond its mechanistic role, P-gp has become a model system for understanding MDR biology, and research focus has shifted toward inhibiting its efflux function, which can fully abrogate MDR in cancer models in vitro. Yet, despite decades of research, the field has struggled to translate P-gp inhibition into clinical benefit (8). Early generations of P-gp inhibitors were limited by toxicity, pharmacokinetic interactions, and insufficient potency (4). These challenges prompted a conceptual shift: instead of attempting to block P-gp function, researchers began exploring ways to exploit P-gp overexpression as a therapeutic vulnerability (9).

This review synthesizes our group's contributions to this evolving landscape. We highlight how P-gp can serve as a therapeutic target and a protector of drug-tolerant persister cells, and how pharmacokinetics and optimized dosing schedules can be leveraged to overcome resistance. We also discuss the emergence of therapy-induced senescence as a reversible survival program and describe how these insights converged in the development of LiPyDau, a next-generation liposomal anthracycline with potent activity against MDR tumors.

These observations reinforce the idea that P-gp is not merely a transporter but a marker of a broader adaptive program. In our own work, this insight helped motivate the search for MDR-selective compounds and shaped our understanding of how P-gp expression defines unique metabolic vulnerabilities that can be therapeutically exploited.

## TURNING THE TABLE ON MDR: P-gp AS A THERAPEUTIC TARGET

Over the past two decades, our understanding of multidrug resistance has undergone a profound conceptual shift. What began as an effort to inhibit P-glycoprotein has evolved into a far more nuanced view in which P-gp overexpression itself becomes a therapeutic vulnerability. This transformation was initiated by our pharmacogenomic analysis of the NCI-60 cancer cell panel, which revealed that while many compounds behave as classical P-gp substrates (recognized and pumped out of the cell instead of accumulating intracellularly), a distinct group displays the opposite pattern, their toxicity is enhanced in P-gp–overexpressing cells (10). This overturned the long-held assumption that P-gp universally protects cancer cells and suggested that MDR creates unique metabolic liabilities.

A major step forward came with the work of Türk et al., who performed a large-scale in silico screen of ~42,000 compounds from the NCI DTP database (11). By correlating drug sensitivity patterns with ABCB1 mRNA expression across the NCI-60 panel, they identified 64 candidate compounds predicted to be more toxic against P-gp overexpressing cells. Experimental validation confirmed 22 robust MDR-selective agents, enriched in three chemotypes, known for their metal chelating properties: isatin-ß-thiosemicarbazones, phenanthroline/bipyridine metal complexes, and 8-hydroxy-quinolines. Crucially, MDR-selective toxicity required functional P-gp, as efflux inhibition with PSC833 abolished hypersensitivity. This study provided the first systematic evidence that P-gp overexpression can be exploited therapeutically and established the chemical space from which later MDR-selective agents emerged.

Building on this foundation, Pape et al. identified additional thiosemicarbazones and related small molecules that preferentially kill MDR cells by exploiting the metabolic burden imposed by chronic P-gp activity (12). These compounds disrupt mitochondrial function and redox homeostasis, triggering selective apoptosis in resistant cells. Our further work expanded this concept by showing that certain MDR-selective compounds not only kill P-gp–overexpressing cells but also downregulate P-gp expression itself, collapsing the MDR phenotype from within and resensitizing cells to standard chemotherapies (13).

The chemical space of MDR-selective agents continued to grow with the work of Cserepes et al. and Pape et al., who mapped structure–activity relationships and identified key molecular features associated with MDR selectivity (14, 15). Their findings demonstrated that MDR-selective compounds can be effective across diverse tumor types, underscoring the broad therapeutic potential of this strategy.

Together, these studies form a coherent narrative that fundamentally reframes MDR biology. P-gp overexpression, long viewed as an insurmountable obstacle, is in fact a double-edged sword. While it protects cancer cells from many chemotherapeutic agents, it simultaneously creates metabolic, signaling, and homeostatic dependencies that can be therapeutically exploited. MDR-selective compounds represent a new class of anticancer agents that turn the logic of resistance on its head: instead of trying to inhibit P-gp, we use its overexpression as a marker and mechanism of selective vulnerability.

## P-gp PROTECTS DRUG-TOLERANT PERSISTER (DTP) CANCER CELLS

In our work published by Szebényi et al. (16), we examined one of the most elusive and clinically consequential phenomena in oncology: the emergence of drug-tolerant persister (DTP) cells following chemotherapy *(Figure 1)*. While classical models of resistance emphasize genetic selection or mutation acquisition, our findings reveal that breast cancer cells can survive high-dose chemotherapy through a reversible, non-genetic adaptation that is mechanistically distinct from stable multidrug resistance. This transient persister state is characterized by cell-cycle arrest, epithelial–mesenchymal plasticity, metabolic rewiring, and, critically, the temporary upregulation of P-gp as a cellular detoxification mechanism.

Using three human epithelial breast cancer cell lines treated with clinically relevant IC30 doses of doxorubicin, cisplatin, or olaparib, we established a robust in vitro model that recapitulates the full trajectory of DTP biology. Within the first days of treatment, cells experienced extensive DNA damage and massive cell death, accompanied by striking morphological changes reminiscent of epithelial-to-mesenchymal transition (EMT). Surviving cells entered a prolonged quiescent state, accumulating in G1 phase and remaining dormant for weeks. Eventually, a subset of these DTPs re-entered the cell cycle and repopulated the culture, demonstrating that the persister state is not terminal but a reversible survival program *(Figure 1a)*.

A key discovery of our study is that P-gp is transiently induced during the DTP state, even in cell lines that do not express P-gp under baseline conditions. This induction was observed after doxorubicin and olaparib treatment, but not after cisplatin, consistent with the substrate specificity of the transporter. Importantly, P-gp expression was restricted to a small subpopulation of persister cells, suggesting a selective survival advantage rather than a global response to stress.

Mechanistically, we found that P-gp plays a crucial role in detoxifying reactive oxygen species (ROS)-induced lipid peroxidation products, which accumulate as a consequence of chemotherapy *(Figure 1b)*. DTP cells face elevated oxidative stress and rely heavily on antioxidant pathways for survival (17, 18). By exporting toxic lipid peroxidation byproducts (19, 20), P-gp reduces intracellular damage and enables cells to withstand the metabolic burden of chemotherapy-induced stress.

This vulnerability became therapeutically actionable. When we inhibited P-gp using the clinically advanced inhibitor tariquidar, the ability of DTP cells to recover and repopulate was almost completely abolished *(Figure 1c)*. Tariquidar did not increase acute cytotoxicity during drug exposure; instead, it prevented long-term survival by blocking the detoxification pathway essential for persister maintenance. This finding highlights a critical distinction: P-gp is not merely a drug efflux pump in this context, it is a survival factor that protects DTPs from oxidative damage.

We extended these observations to an in vivo setting using the Brca1-/-;p53-/- genetically engineered mouse model of hereditary triple-negative breast cancer (21). In this clinically relevant system, prolonged administration of tariquidar during chemotherapy "drug holidays" significantly improved overall survival without exacerbating toxicity *(Figure 1c)*. This result suggests that targeting DTP-specific vulnerabilities during off-treatment periods may be a viable strategy to delay or prevent relapse. As the lipid translocase function of P-gp is cell type independent (19, 20), we expect this mechanism to apply across different tumor types beyond breast cancer, where P-gp is induced in response to treatment or inherently expressed.

Our study positions DTPs as a central, reversible reservoir of therapy resistance and identifies P-gp–mediated detoxification as a key mechanism supporting their survival. These insights bridge the conceptual gap between transient drug tolerance and stable multidrug resistance, showing how short-term adaptations can seed long-term therapeutic failure. By demonstrating that P-gp inhibition selectively disrupts the persister state, we provide a rationale for revisiting transporter-targeted therapies, not as co-treatments during chemotherapy, but as strategic interventions during recovery phases when DTPs are most vulnerable.

Together, these findings deepen our understanding of non-genetic resistance programs and highlight new therapeutic opportunities to eliminate the persister reservoir that fuels relapse in breast cancer.

## A NOVEL DRUG RESISTANCE MECHANISM: THERAPY-INDUCED SENESCENCE

Therapy-induced senescence (TIS) has traditionally been regarded as a beneficial outcome of anticancer therapy: a stable, irreversible arrest that prevents damaged tumor cells from re-entering the cell cycle (22). However, our recent work fundamentally challenges this long-standing assumption. In Bajtai et al. (23), we demonstrate that TIS is not a terminal fate but a dynamic, reversible, and biologically purposeful state that enables tumor cells to survive chemotherapy and later drive relapse *(Figure 2a–c)*.

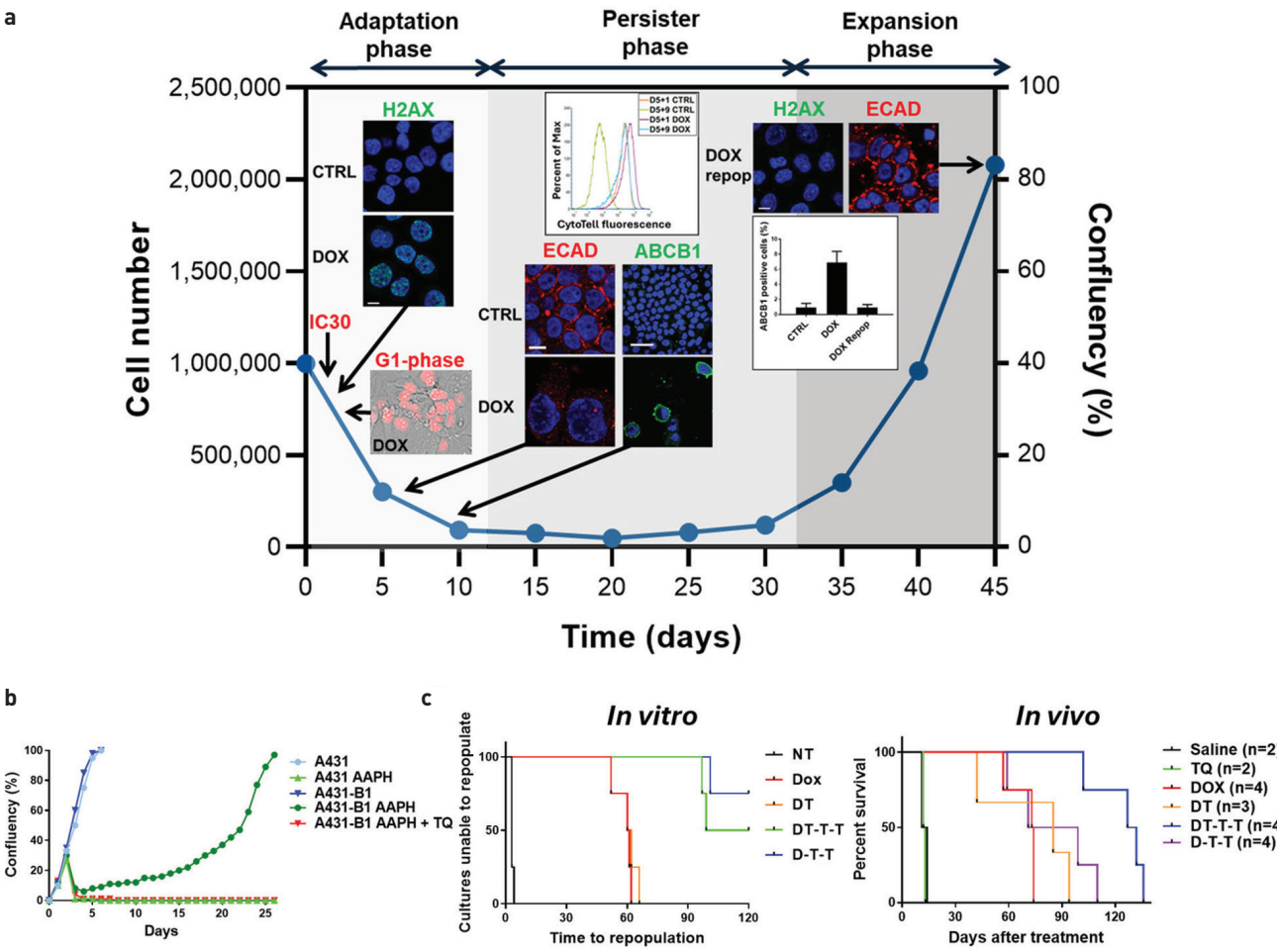


**FIGURE 1.** Cellular, metabolic, and survival features of drug-tolerant persister cells. a) Schematic overview of the in vitro assay used to study the drug-tolerant persister (DTP) state. Cells were treated with IC30 doxorubicin (DOX) for 5 days, after which surviving cells were cultured in drug-free medium. DTP cells exhibit extensive DNA damage 24 h after treatment (phospho-H2AX puncta; scale bar: 10 μm), EMT-like changes after 5 days (internalization of E-cadherin; scale bar: 10 μm), and upregulation of ABCB1/P-gp by day 7 (scale bar: 50 μm). Growth arrest during the persister phase was confirmed using a G1-phase cell-cycle reporter and CytoTell label-dilution assays (baseline fluorescence recorded at D5+1; DOX-treated vs. control samples compared at D5+9). Repopulating cells resemble pre-treatment controls in phospho-H2AX levels, E-cadherin localization, and P-gp positivity. Nuclei were stained with DAPI (blue). b) Repopulation assay of untreated (blue) and AAPH-treated cells with or without the P-gp inhibitor tariquidar (TQ), demonstrating that P-gp supports survival by exporting toxic lipid peroxidation products. A431 (light green) and A431-B1 (dark green) cells were treated with 10 mM AAPH for 48 h and allowed to recover in the absence or presence of TQ (red). c) Transient induction of P-gp is essential for long-term survival after chemotherapy. In vitro repopulation curves show recovery times following different treatment protocols: NT (untreated), DOX (5-day DOX), DT (DOX+TQ for 5 days), DT-T-T (DOX+TQ for 5 days followed by two 3-day TQ treatments), and D-T-T (DOX for 5 days followed by two 3-day TQ treatments). In vivo survival curves from a clinically relevant breast cancer model show improved outcomes when TQ is administered with DOX (DT), maintained for 5 additional days each cycle (DT-T-T), or applied after DOX (D-T-T). Treatment cycles were repeated when tumors returned to baseline volume. Figure panels were taken from our previous publication (16), licensed under CC BY 4.0.

Using a combination of bulk and single-cell transcriptomics *(Figure 2d),* live-cell imaging, and functional assays, we show that breast cancer cells exposed to clinically relevant doses of chemotherapy enter a senescent-like state marked by growth arrest, persistent DNA damage signaling, and extensive transcriptional rewiring. Importantly, single-cell RNA sequencing revealed that these TIS cells do not arise from a pre-existing subpopulation, but instead emerge directly in response to therapy, indicating that senescence induction is a widespread and adaptive response rather than the selection of a rare, pre-determined cell fraction. Crucially, this state is not fixed. Over time, a subset of these senescent cells, what we term escapers, reactivate proliferation and give rise to a population that is phenotypically and transcriptionally distinct from both the original tumor cells and the senescent population *(Figure 2e).*

One of the most striking findings of our study is that escape from TIS is not a rare stochastic event. Instead, it is a robust, reproducible, and biologically regulated process observed across multiple breast cancer models. The escapees exhibit a unique transcriptional signature characterized by altered cell-cycle control, metabolic reprogramming, and activation of stress-response pathways. These changes are not passive consequences of chemotherapy; they actively endow the cells with enhanced survival capacity and a greater ability to repopulate the tumor mass once treatment pressure is removed.

We also uncover that TIS escape is accompanied by deep chromatin remodeling, including changes in heterochromatin organization and the expression of chromatin-associated proteins. These structural alterations likely provide the plasticity required for cells to transition between senescence, quiescence, and renewed proliferation. In parallel, inflammatory and SASP-related pathways remain active during the escape process, potentially shaping a microenvironment that supports tumor regrowth.

Perhaps the most clinically relevant insight from our work is the recognition that TIS represents a previously underappreciated drug-resistance mechanism. Unlike classical resistance, which is often driven by stable genetic mutations, TIS-mediated resistance is non-genetic and reversible. This distinction has profound implications. It suggests that even tumors lacking pre-existing resistant clones can survive therapy by entering a transient senescent state, only to relapse once escape occurs. This mechanism may help explain why many patients experience recurrence despite initial therapeutic responses and in the absence of detectable resistance mutations.

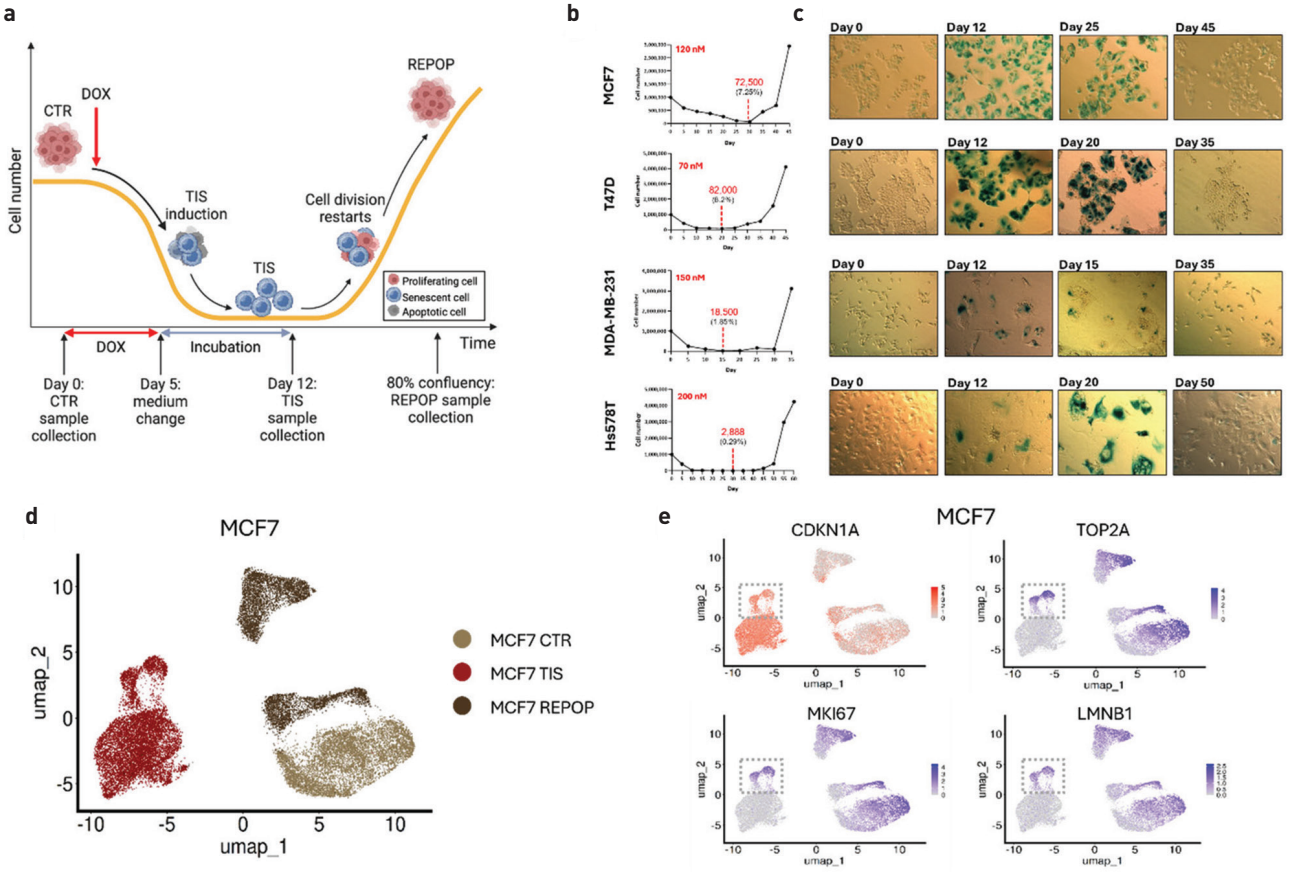


**FIGURE 2.** Cellular and transcriptional features of therapy-induced senescence and repopulation. a) Schematic overview of the assay used to study therapy-induced senescence (TIS) and subsequent repopulation (REPOP). b) Growth kinetics of breast cancer cells following 5-day high-dose doxorubicin (DOX) treatment. DOX was applied on day 0 and removed on day 5; minimal cell numbers are indicated in red. c) SA-β-Gal activity detected by X-gal staining in control (CTR), TIS, and REPOP cells. d) UMAP of MCF7 single-cell transcriptomes showing distinct clustering of CTR (light brown), TIS (scarlet), and REPOP (dark brown) populations, with REPOP partially shifting toward CTR, indicating reversal of the TIS-associated transcriptional program. e) Feature plots of selected genes: TIS cells show high CDKN1A (p21) expression, indicative of cell cycle arrest, and reduced proliferation markers (LMNB1, TOP2A, MKI67). A subset of TIS cells marked with dotted regions ("escapers") displays increased proliferation-associated gene expression, suggesting potential cell cycle re-entry. Figures were taken from our previous publication (23), licensed under CC BY 4.0.

Our findings also raise important questions about the therapeutic use of senolytics, agents designed to selectively eliminate senescent cells (24). While senolytics have been proposed as a strategy to eradicate TIS cells and prevent relapse, we show that currently available senolytic agents fail to efficiently eliminate TIS cells in vivo. This underscores the need for next-generation senolytics tailored specifically to the biology of therapy-induced senescence, rather than relying on agents developed for aging-associated senescence.

Beyond the mechanistic insights, our study reframes how we understand tumor response to therapy. Rather than viewing senescence as a protective endpoint, we position TIS as a transitional state that can serve as a reservoir for relapse. This perspective aligns with emerging evidence from drug-tolerant persister biology, EMT plasticity, and stress-induced dormancy. Together, these fields reveal that cancer cells possess a remarkable ability to adopt reversible, protective phenotypes that allow them to withstand therapeutic stress.

It is important to mention that though DTPs and senescent cells share common traits, they are different cellular states, based on the duration of their dormant state, secretory phenotype and related influence on tumor microenvironment.

## PHARMACOKINETICS AND DOSING SCHEDULES AS TOOLS AGAINST DRUG RESISTANCE

Our work over the past decade has repeatedly demonstrated that drug resistance is not solely dictated by tumor-intrinsic biology. The way a drug is delivered, its pharmacokinetic profile, its exposure dynamics, and the temporal pattern of dosing, can decisively shape therapeutic outcomes. Two of our studies, separated by nearly a decade, illustrate this principle from complementary angles: the pharmacokinetic optimization of doxorubicin through liposomal encapsulation (25) and the algorithm-assisted personalization of chemotherapy schedules (26). Together, these findings highlight that resistance can be delayed, mitigated, or even overcome by manipulating exposure rather than molecular targets.

In our 2017 study, we examined why pegylated liposomal doxorubicin (PLD) performs dramatically better than conventional doxorubicin in the Brca1-/-;p53-/- genetically engineered mouse model of hereditary breast cancer (25). Although PLD remains susceptible to P-glycoprotein–mediated efflux in vitro, its in vivo behavior is fundamentally transformed by its altered pharmacokinetics. Liposomal encapsulation increases the maximum tolerated dose, prolongs circulation time, and produces a ~2600-fold increase in area under the curve compared to free doxorubicin. As a result, PLD maintains high plasma levels for days rather than minutes, enabling sustained tumor exposure through the enhanced permeability and retention effect. This prolonged exposure translates into markedly improved relapse-free and overall survival, even in tumors that have already acquired resistance to conventional doxorubicin. Importantly, PLD does not bypass efflux at the cellular level; instead, it overwhelms resistance mechanisms by maintaining continuous cytotoxic pressure, preventing tumor cells from exploiting pharmacokinetic gaps to recover and adapt.

Nearly a decade later, in our 2026 study, we approached the same problem from the opposite direction: rather than modifying the drug, we modified the dosing schedule (26). Using the same Brca1-/-;p53-/- model, we developed an algorithm-assisted therapy design (AATD) framework that personalizes chemotherapy based on measurable biological parameters such as tumor growth kinetics and drug pharmacokinetics. Two individualized strategies were implemented: a pharmacokinetically driven protocol (PDPK) designed to maintain stable, low plasma concentrations of PLD, and a model-predictive control (MPC) approach that dynamically adjusts dosing based on real-time tumor behavior. Both strategies significantly outperformed conventional maximum-tolerated-dose therapy, which relies on large, intermittent boluses administered only after tumor relapse. These long drug-free intervals create ideal conditions for tumor cells to enter reversible survival states, accumulate adaptive changes, and eventually develop resistance.

In contrast, AATD protocols smooth the exposure curve, eliminate drug-free windows, and tailor dose intensity to the tumor's actual dynamics. This approach reduces relapse frequency, delays the emergence of resistance, and prolongs overall survival—often with lower cumulative doses. The success of AATD underscores a critical insight: resistance is frequently a consequence of suboptimal scheduling, not just cellular evolution. By reshaping the temporal pattern of drug delivery, we can alter the selective pressures that drive resistance and maintain therapeutic control for far longer than with standard regimens.

Accurate determination of tumor size in future applications may be achieved through more frequent imaging modalities, such as computed tomography (CT) and magnetic resonance imaging (MRI). In addition, monitoring of key biomarkers can be performed using blood-based assays, including the analysis of circulating tumor DNA (ctDNA). Further developments may focus on innovative technologies that enable the rapid and precise quantification of drug concentrations from minimal sample volumes, potentially even from a single drop of blood.

Viewed together, these two studies converge on a unifying principle: exposure matters as much as mechanism. PLD succeeds because it changes where and for how long doxorubicin is present. AATD succeeds because it changes when and how much drug is delivered. Both strategies demonstrate that resistance can be countered not only by developing new drugs, but also by optimizing the pharmacokinetic and temporal dimensions of existing ones. This perspective reframes resistance as a pharmacological and mathematical challenge as much as a biological one, opening new avenues for therapeutic innovation without requiring new molecular entities.

## A PROMISING NEW ANTICANCER FORMULATION: LiPyDau

In our recent work on LiPyDau (27), we set out to address one of the most persistent challenges in anthracycline-based chemotherapy: how to retain the extraordinary antitumor potency of this drug class while overcoming its dose-limiting toxicities and its propensity to induce therapy resistance. Anthracyclines remain foundational in the treatment of breast cancer and many other malignancies, yet their clinical utility is constrained by cardiotoxicity, myelosuppression, and the rapid emergence of resistance. Our goal was to engineer a compound that preserves the cytotoxic strength of anthracyclines while fundamentally altering their pharmacological behavior.

LiPyDau represents the culmination of this effort. It is a liposomal formulation of a daunosamine-modified anthracycline derivative, designed to combine the chemical potency of the parent molecule with the pharmacokinetic advantages of liposomal delivery *(Figure 3)*. The daunosamine modification dramatically increases intrinsic cytotoxicity and evades P-gp efflux, but the free compound is too toxic for systemic administration. Encapsulation in a PEGylated liposomal carrier solves this problem, enabling safe delivery of an otherwise unusable drug *(Figure 3a,b)*.

Across multiple preclinical models, including orthotopic, genetically engineered, and metastatic settings, we observed that LiPyDau induces complete and durable tumor regression, often with a single treatment cycle *(Figure 3c)*. These responses were not only deeper than those achieved with conventional doxorubicin or PLD, but also markedly more sustained. In several models, tumors failed to relapse even after extended follow-up, suggesting that LiPyDau triggers a fundamentally different biological response than classical cytotoxic agents.

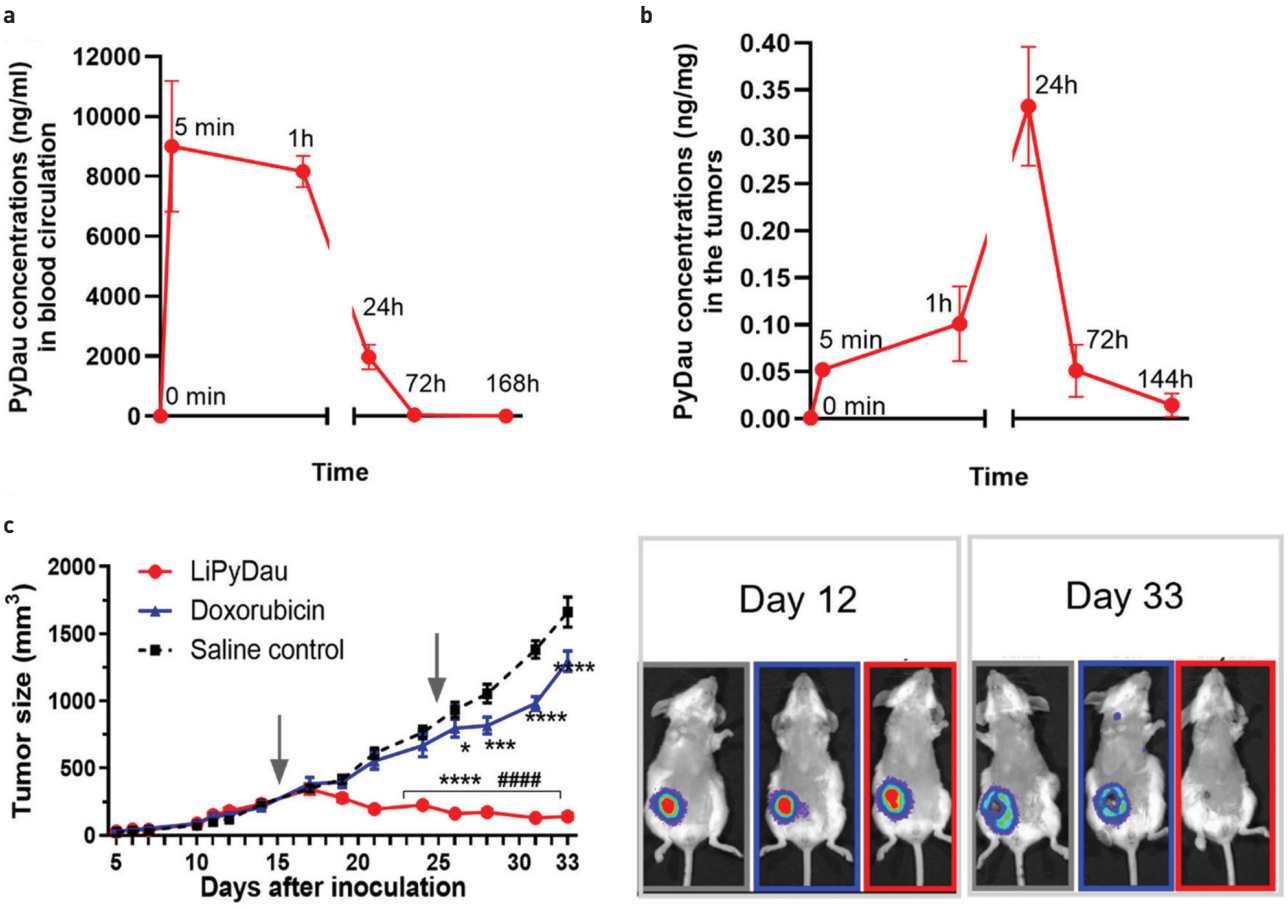


**FIGURE 3.** Pharmacokinetics and antitumor efficacy of LiPyDau. Serum (a) and intratumoral (b) PyDau concentrations in mice following a single 1 mg/kg intravenous dose of LiPyDau. PyDau levels were quantified at the indicated time points by mass spectrometry. Data represent means of triplicate aliquots from three mice per time point; error bars indicate ± SD. (c) Tumor growth response of orthotopically transplanted, highly aggressive 4T1-LUC breast cancer cells. Whereas doxorubicin treatment (blue) produces only minimal tumor control, LiPyDau (red) achieves robust and sustained suppression of tumor growth. Figure panels, licensed under CC BY 4.0, were taken from our previous publication (27) with slight modifications.

A central mechanistic insight from our study is that LiPyDau's therapeutic profile is shaped by the combined effects of its chemical modification and its liposomal pharmacokinetics. The formulation achieves high and sustained intratumoral concentrations while maintaining acceptable systemic exposure, enabling prolonged cytotoxic pressure without the toxicity associated with free anthracyclines. This pharmacological behavior provides a plausible explanation for the exceptional tumor control observed in vivo and highlights how rational drug design can overcome classical resistance mechanisms without requiring transporter inhibition or combination therapy.

Pharmacokinetic analyses revealed that liposomal encapsulation enables prolonged systemic exposure and high tumoral accumulation of the active compound, far exceeding what is achievable with free anthracyclines. Importantly, this enhanced exposure does not translate into increased systemic toxicity. LiPyDau was well tolerated across all tested models, with no evidence of cardiotoxicity or severe off-target effects. This safety profile is particularly notable given the extraordinary potency of the encapsulated drug.

One of the most compelling findings of our study is that LiPyDau remains effective in anthracycline-resistant tumors, including models with high P-gp expression and prior doxorubicin failure. This distinguishes LiPyDau from PLD and other liposomal formulations, which remain susceptible to efflux-mediated resistance. The combination of chemical modification and liposomal delivery appears to fundamentally alter how tumor cells process and respond to the drug.

Taken together, our results position LiPyDau as a next-generation anthracycline with the potential to overcome long-standing limitations of this drug class. By integrating chemical innovation with optimized delivery, LiPyDau achieves deep and lasting tumor control while avoiding the toxicities that constrain conventional anthracyclines. Its efficacy in resistant tumors further underscores its translational promise.

In the broader context of therapy resistance, LiPyDau provides a striking contrast to the reversible survival states described in our other studies. Whereas standard chemotherapies often push tumor cells into transient adaptive programs that ultimately fuel relapse, LiPyDau appears to enforce a therapeutic response that prevents recovery. This unique profile makes it an attractive candidate for future clinical development and a powerful example of how rational drug design can reshape therapeutic outcomes.

Next steps for the development of LiPyDau include evaluation in higher animal models, completion of standardized toxicity studies, and the development of scalable manufacturing processes to support clinical translation. In parallel, its antitumor activity should be further explored across malignancies, including indications with poor prognosis or limited therapeutic options, as well as settings where clinical liposomal anthracyclines (Myocet, PLD) are not currently used. These efforts will provide the foundation for early-phase clinical trials and help define the clinical contexts in which LiPyDau may offer meaningful therapeutic benefit.

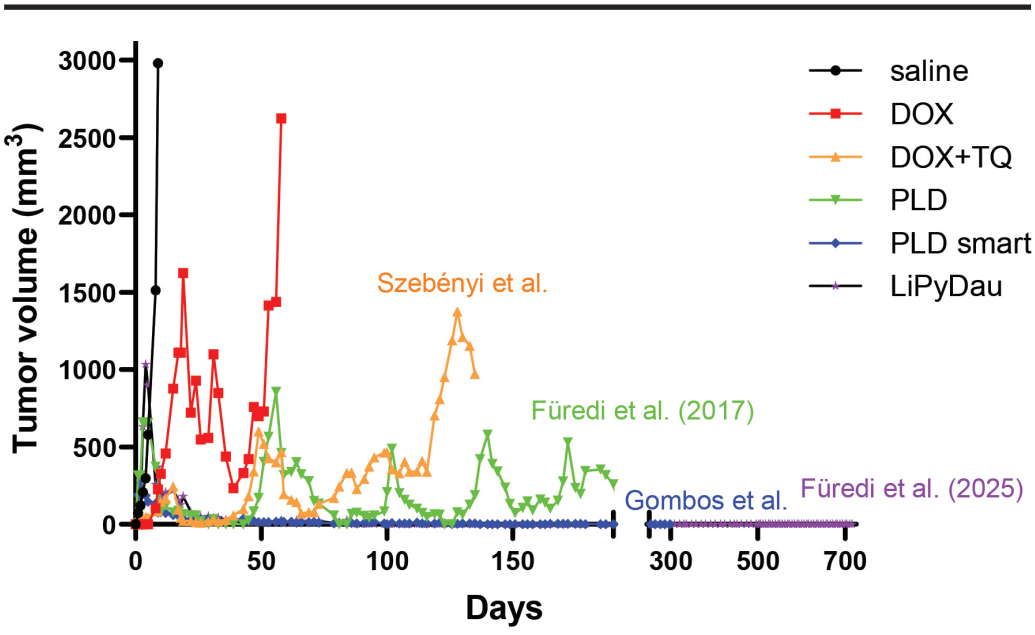


**FIGURE 4.** Stepwise therapeutic innovations progressively extend survival in the Brca1/p53-KO breast cancer model. Tumor growth kinetics of Brca1/p53-KO mice treated with successive therapeutic strategies.

## FROM CYTOTOXICITY TO RATIONAL DESIGN: HOW MECHANISTIC INSIGHT EXTENDED SURVIVAL TEN-FOLD

Over the past decade, our work in the Brca1/p53-KO hereditary breast cancer model has demonstrated how rational, mechanism-guided innovation can progressively and dramatically extend survival *(Figure 4).* Starting from the limited efficacy of doxorubicin monotherapy, which yields median survival of only 60–70 days, each successive strategy we developed produced a measurable and biologically meaningful improvement. Targeting transient P-gp–mediated detoxification with the DOX+TQ combination doubled survival to ~130 days [Szebényi et al. (16)]. Modifying pharmacokinetics through pegylated liposomal doxorubicin further extended survival to ~200 days [Füredi et al., 2017 (13)], while algorithm-assisted therapy design (AATD) of PLD pushed this boundary to nearly 300 days by eliminating drug-free intervals and reshaping exposure dynamics [Gombos et al. (26)]. Most recently, LiPyDau achieved near-complete long-term survival approaching 700 days, representing a transformative leap in therapeutic durability [Füredi et al., 2025 (27)]. Together, these advances illustrate a coherent trajectory in which mechanistic insight, pharmacological engineering, and adaptive scheduling converge to overcome resistance and redefine what is achievable in aggressive, treatment-refractory breast cancer.

## CONCLUSIONS

Cancer drug resistance is often portrayed as an inevitable consequence of tumor evolution, but the body of work summarized here demonstrates that resistance is neither monolithic nor insurmountable. By dissecting its molecular, cellular, and pharmacokinetic foundations, we reveal that resistance emerges from a series of reversible survival programs, from transporter-driven efflux to drug-tolerant persisters and therapy-induced senescence, each governed by distinct vulnerabilities that can be strategically targeted.

Our studies show that P-gp overexpression, long viewed as a therapeutic obstacle, can be transformed into a liability through MDR-selective compounds. We demonstrate that persister cells, once considered an obscure in vitro artifact, represent a clinically relevant reservoir of relapse that can be eliminated by disrupting P-gp-mediated detoxification. We reveal that TIS is not a terminal fate but a dynamic, escape-prone state that fuels recurrence. Furthermore, we show that pharmacokinetics and dosing schedules are powerful, underappreciated determinants of therapeutic success, capable of reshaping tumor evolution when optimized through liposomal formulations or algorithm-assisted therapy design.

Finally, the development of LiPyDau illustrates how these insights can converge into a new generation of anticancer agents, compounds that combine rational chemistry, optimized delivery, and an understanding of tumor plasticity to achieve deep and durable responses even in resistant settings.

Together, these advances point toward a new paradigm: drug resistance is not a single problem with a single solution, but a landscape of transient states that can be intercepted, exploited, or permanently shut down. By embracing this dynamic view, we can design therapies that not only overcome resistance but prevent its emergence. The future of oncology lies in integrating molecular mechanisms with pharmacological innovation, transforming resistance from an inevitable barrier into a solvable challenge. Understanding and targeting these transient states may define the next decade of precision oncology.

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