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Drug-tolerant persister cells in cancer: bridging the gaps between bench and bedside

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Drug-tolerant persister (DTP) cells represent a major obstacle to achieving durable cancer remission, yet their biology and clinical relevance remain poorly understood. This perspective highlights key gaps hindering the translation of DTP research into clinical progress, emphasizing the need to move beyond reductionist models toward integrative, patient-aligned approaches that reflect clinical complexity. Bridging these divides will be crucial to reveal actionable biomarkers and develop therapies capable of eradicating these resilient cell populations.

Drug-tolerant persister cells represent a rare but deadly survivor subpopulation within cancer malignancy[1]. They are a small fraction of cancer cells that survive standard-of-care (SOC) therapies not through stable genetic resistance, but via reversible, non-genetic adaptations[2]. Acting as clinically occult reservoirs, DTP cells persist in the shadows, seeding relapse long after the visible tumour has regressed[3]. We highlight an urgent insight: that the true danger in cancer may not always be what is visible and rapidly growing, but what is silently enduring and unacknowledged. Overcoming this threat requires a paradigm shift: from focusing solely on eradication of bulk tumour cells to targeting the hidden reservoir of persistence.

Inspired by the concept of bacterial persisters first described by Bigger[4], Sharma et al. identified these reversible, drug-tolerant cancer cells in EGFR-mutant non-small cell lung cancer (NSCLC) models treated with EGFR inhibitors in 2010[5]. Since then, interest in DTP biology has grown rapidly, revealing a spectrum of adaptive traits, epigenetic reprogramming[6], transcriptional memory[7], translational remodelling[8], metabolic shifts[9,10] and therapy-induced mutagenesis[11], across diverse tumour types and treatments. Despite these advances, most DTP studies to date have relied heavily on in vitro or ex vivo models, limiting their physiological relevance. Recent efforts have begun to explore minimal residual disease in vivo, including through patient-derived xenografts (PDXs)[12,13], but these models often lack immune components and do not capture the broader systemic influences, such as patient age, sex, metabolic state, or organ-specific macroenvironments, that may critically shape DTP behaviours[14]. Moreover, DTPs engage diverse adaptive programs that transcend genetically determined lineages and vary in response to different treatment strategies, allowing them to concomitantly adopt multiple phenotypes. This has been observed across tumour types. For instance, single-cell RNA sequencing (RNA-seq) has shown that DTPs with mesenchymal-like and luminal-like transcriptional states can coexist within breast cancers[15]. Similarly, pioneering studies in melanoma treated with BRAF inhibitors demonstrated that multiple phenotypic states could coexist within DTP populations[16]. Recent work integrating single cell molecular profiling with DNA barcoding lineage tracing has further revealed that genetically similar cancer cells can diverge into distinct clonal fates after treatment[17]. These fates are not fixed and can shift depending on treatment dose and type, highlighting that variability in intrinsic cell states may represent a general feature of DTP responses. This inherent plasticity and heterogeneity make it difficult to define common vulnerabilities across cancer types and treatment contexts, thereby reinforcing a persistent bench-to-bedside gap, in which DTPs remain clinically under-characterized, methodologically elusive, and largely excluded from mainstream drug development pipelines.

The DTP-related adaptive mechanisms driving response, tolerance, and resistance to specific treatment regimens, such as radiation, targeted therapy, chemotherapy, and immunotherapy, indeed differ across tumour types. Readers interested in these therapy- and tumour-

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specific mechanisms are referred to other comprehensive reviews[1–3]. In contrast, the purpose of this perspective is not to catalogue mechanisms in a treatment- or cancer type–dependent manner, but rather to highlight coherent emerging themes that cut across systems and point to future directions in DTP biology. These include clarifying how DTPs relate to other cancer cell states, defining the mechanisms that govern their emergence, persistence, and reactivation during therapy, and moving from isolated observations to integrated models that incorporate microenvironmental cues, organ specificity, and host physiology. Building on these themes, we also discuss transformative strategies with the potential to reshape both the study and clinical translation of DTPs. By focusing on these cross-cutting questions, we aim to inspire deeper mechanistic exploration and a more integrated, clinically oriented research direction.

Every identity is made of difference

DTPs share several cardinal features with a spectrum of cancer cell states and phenotypes, including dormant disseminated tumour cells (DTCs)[18], cancer stem cells (CSCs)[19], senescent cells[20] and drug-induced cytostasis[21] (Table 1). Despite these similarities, the biological and phenotypic characteristics of DTPs remain less clearly defined in the literature. This likely reflects the substantial heterogeneity in the field, both in terms of specificity and generalizability of findings. Mechanistic distinctions between these cell states are often context-dependent, shaped by cell-intrinsic factors, therapeutic pressures, and the tissue microenvironment.

Tumour dormancy, originally conceptualized in the 1950s as a reversible mitotic arrest[22], comprises three categories: cellular, angiogenic, and immune-mediated dormancy. Cellular dormancy involves quiescent DTCs. Angiogenic dormancy stems from poor vascularization, while immune-mediated dormancy results from sustained immune pressure—both involving macroscopic tumour masses, unlike the microscopic dormancy of DTCs and DTPs. Though DTPs share some traits with DTCs, they differ in key aspects. DTCs are typically Ki67-negative[23] and survive in niche-dependent states. DTPs, by contrast, are exclusively induced by SOC therapy and show heterogeneous phenotypes, including both quiescent and slow-cycling cells[24]. In breast cancer, hypoxia in the primary tumour can prime subsets of cells into dormancy via NR2F1 and SOX9 expression. Stromal cells like macrophages and endothelial cells help sustain this state in metastatic niches[25]. Whether DTPs are similarly primed across contexts is unclear. In triple-negative breast cancer, a similar pre-DTP state has also been observed, where a subset of tumour cells exhibits bivalent chromatin configurations prior to treatment with the DNA synthesis inhibitor capecitabine, predisposing them to acquire a DTP phenotype[26]. Unlike DTCs, this priming is niche-independent and can arise stochastically over time, as observed in HER2+ breast cancer under lapatinib treatment[15]. Beyond breast cancer, for instance, in melanoma, stochastic transcriptional heterogeneity appears to enable the emergence of a niche-independent pre-DTP state upon exposure to BRAF-targeted therapy[27]. Moreover, DTP states are not limited to pre-existing clones, as they can emerge reproducibly from genetically identical single cells[5,28], indicating that priming likely reflects stochastic transcriptional variation rather than fixed subclones. Both DTCs and DTPs can evade immune surveillance, but their mechanisms may differ. DTC escape detection due to its scarcity, as MHC-I downregulation does not prevent recognition by engineered T-cell receptor T cells[29]. In contrast, DTPs in osimertinib-treated EGFR mutant NSCLC upregulate CD70 via promoter demethylation[30], promoting both survival and immune evasion by engaging CD27 on immune cells.

CSCs, unlike DTPs, are a small population capable of asymmetric division and differentiation, contributing to tumour initiation and therapy resistance[19]. They express markers such as CD44, CD133, ALDH, CD24 or CD166, depending on tissue type[31]. Colorectal cancer often consists of both LGR5^{+} proliferative CSCs, enriched in β-catenin, MAPK and MYC signalling[32], and LGR5^{-}Annexin A1^{+} slow-cycling CSCs, which show FAK/YAP[33] and inflammatory signalling profiles[34]. In colorectal cancer patient-derived organoids (PDOs), chemotherapy-induced DTPs resemble slow-cycling CSCs, mediated by MEX3A-dependent deactivation of the WNT pathway through YAP1[35]. The colorectal DTPs upon exposure to FOLFOX (5-fluorouracil/leucovorin + oxaliplatin) also undergo oncofetal-like reprogramming, entering a diapause-like state, a trait shared with LGR5^{-} slow-cycling CSCs[36]. Retinoid X receptor dysfunction appears to act as a gatekeeper for this lineage plasticity, establishing a persistent oncofetal-like "memory" maintained by YAP/AP-1[37]. A similar oncofetal-like cell state has been also observed in triple-negative breast cancer, where DTPs induced by neoadjuvant chemotherapy can adopt a fetal-like alveolar progenitor state, marked by FXYD3 expression, resulting in drug tolerance[38]. Because DTP survival programs vary with treatment and cancer type, whether this oncofetal-like spectrum is a universal feature of therapy-induced DTPs across cancer types remains to be fully defined.

Cellular senescence is a stress-induced, growth-arrested state often accompanied by senescence-associated secretory phenotype (SASP)[20]. DTPs share some features with senescent cells, including reversible arrest, metabolic reprogramming, and paracrine activity, but lack consistent senescence markers. In Sharma's EGFR mutant NSCLC DTP model, γH2AX was absent upon EGFR-targeted therapy exposure, unless HDAC inhibition was applied, triggering caspase-independent cell death[5]. Similarly, in PDOs from metastatic colorectal cancer, Punzi et al. observed that colorectal DTPs exposed to FOLFOX displayed low γH2AX levels and reduced CHK1/CHK2 activity[39]. The role of p21 and p16^{INK4a}, classical senescence markers, remains also ambiguous in DTPs. Using a CRISPR-based p21 reporter, Hsu et al. showed that p21 dynamics during the cell cycle influence cell fate after doxorubicin exposure in NSCLC cell lines, either promoting

Table 1 | Operational distinctions among different phenotypic tumour cell states

	Cytostasis	DTP	DTC	CSC	Senescence
Cell fraction	Whole population	Rare subset	Single cell or small subset	Subset (context-dependent)	Variable (often large fractions)
Growth	Quiescent	Slow-cycling or quiescent	Quiescent, Ki67 negative	Self-renewing	Quiescent
Treatment requirement	Induced by sublethal treatment	Induced by lethal treatment	No	No	Context-dependent
Genetic dependency	No	No	Partial	Partial	Partial
Cell state reversibility	Transient	Yes, upon drug removal	Yes	Yes	Irreversible
Immune evasion	Minimal	Therapy-induced mechanisms	Scarcity dependent	Stemness mechanisms	SASP-modulated immune response
Paracrine activity	Minimal	Yes	Yes	Yes	Exclusively SASP
Niche dependency	No	Low	High	High	Moderate

senescence or allowing recovery, emphasizing the role of treatment timing[40]. At the secretome level, SASP expression also shows treatment-dependent variability: present in NSCLC DTPs following EGFR/MEK inhibitor treatment[41], but absent after mTOR inhibition in the same tumour type[42].

Caution should be taken not to conflate treatment-induced cytostasis or quiescence with true DTPs. SOC therapies, especially targeted inhibitors, often trigger broad, non-proliferative arrest across the bulk tumour population at non-lethal doses or short exposures[21]. For instance, BRAF inhibitors comonly induce cytostatic arrest in BRAF-mutant melanoma and colorectal cancer[43,44], a state that can sometimes precede apoptosis depending on pro-apoptotic mediators such as BCL2-interacting killer[45]. In contrast, DTPs are operationally defined as a rare subset that withstands otherwise lethal drug exposure, representing a distinct survival state rather than a transient, drug-imposed cytostasis. After 15 years of progress since Sharma's work, the question remains: are DTPs, DTCs, CSCs, and senescence different names for the same target?[46] The debate may not be so lasting at last considering that DTPs are uniquely induced by SOC treatment in terms of treatment type, intensity, frequency, and duration, yet much remains to be explored due to their heterogeneous and dynamic nature, a topic we discuss in the next sections.

The known unknowns—unanswered questions in DTP biology

While minimal residual disease is clinically recognized, the cellular mechanisms underlying treatment persistence remain incompletely understood. Diverse survival strategies of DTP cells have been described in various cancer types, yet the coherent molecular mechanisms governing their emergence, maintenance, and reactivation across treatment and tumour types remain a critical and unresolved frontier, essential for bridging the gap between bench and bedside.

What the drug leaves behind: the quiet birth of persistence

The emergence of DTP cells involves both intrinsic and extrinsic mechanisms and unfolds dynamically over time. Heterogeneity in treatment duration across studies blurs distinctions between early adaptive responses and later survival programs, complicating comparisons and masking the temporal evolution of drug tolerance, even under the same therapeutic context[47]. A recent study in *BRCA2*-deficient high-grade serous ovarian cancer cells demonstrated that non-genetic resistance arises as a continuum, involving stepwise transcriptional reprogramming and epigenetic enforcement under escalating doses of the PARP inhibitor olaparib. Notably, DTP cells isolated after 9-day treatment with either 10 µM or 320 µM olaparib recapitulate early cell states along this resistance trajectory[48]. These findings raise a key unresolved question: what intrinsic or extrinsic factors govern the bifurcation between cell death and persistence?

One possible intrinsic factor is sublethal activation of cell death pathways. Kalkavan et al. showed that lung adenocarcinoma cells upon exposure to BH3 mimetics can survive sublethal cytochrome c release by activating the integrated stress response (ISR) transcription factor ATF4[49]. This failed apoptosis, termed anastasis[50], refers to survival from transient apoptotic stimuli, including direct caspase activation. Interestingly, in an optogenetically controlled caspase-3 activation model in HeLa cells, neither the rate, peak, nor total caspase activity predicted cell survival[51]. Although this experiment was not performed in a DTP context, it suggests that intrinsic cell state information may influence outcomes following drug-induced death pathway activation[52]. In the DTP context, one such state-defining feature may be the cell cycle position at treatment onset. In MYCN-high neuroblastoma, cells newly entering G1 arrest at treatment initiation tolerated doxorubicin[53]. This cell-cycle effect may reflect an evolutionarily conserved stress response shared with non-tumoral cells. Using the non-transformed human epithelial cell line MCF10A, Min et al. showed that slow-cycling cells with prolonged G1, identified by CDK2 and p21 activity, exhibited elevated ISR and p53 signalling, priming them for survival under stress conditions[54] (Fig. 1a).

The ISR has also been implicated in rapid drug escape in a subpopulation of melanoma cells treated with BRAF-targeted therapy, via upregulation of ATF4, consistent with the observation from Kalkavan et al.[49]. Whether ISR generally initiates persistence programs upon SOC treatment remains to be evaluated across cancer and treatment types (Fig. 1a). Nevertheless, it was shown that multiple kinase inhibitors activate ISR in various models[55]. Neratinib, a pan-HER kinase inhibitor, and FGFR kinase inhibitor dovitinib both activate ISR upstream kinase GCN2 by directly increasing its ATP affinity. This may represent a general off-target effect of targeted therapy. Ryland et al. found that the BRAF inhibitors dabrafenib and encorafenib directly bind GCN2, rapidly activating the ISR and ATF4 in BRAF-mutant melanoma cells, a response that was similarly observed in EGFR-mutant PC9 cells upon erlotinib exposure[56]. Similar effects were observed in small cell lung cancer (SCLC) treated with WEE1 kinase inhibitor AZD1775, which also activates GCN2 within hours[57]. Thereby, longitudinal and single-cell studies of early responses to SOC treatment, before bona fide DTPs emerge, may illuminate how DTPs are born[58].

In clinically relevant settings, stress response and cell death signals are also shaped by extrinsic cues, such as spatial position within the tumour, proximity to vasculature, and stromal or immune interactions. Analogous to bacterial persistence, microenvironmental context matters: in *Salmonella*, tissue-like nutrient deprivation revealed that antibiotic tolerance was largely driven by starvation[59]. Similarly, cancer cells often reside in nutrient-limited niches. Tumour-relevant low-glucose conditions protect acute T-cell leukaemia cells from chemotherapy by preserving pyrimidine pools through inhibition of UDP-glucose shunting[60]. Glucose restriction also impedes mitochondrial outer membrane permeabilization, possibly leading to anastasis[49,60]. In solid tumours, glutamine starvation can also induce tolerance to the multikinase inhibitors sorafenib and donafenib in hepatocarcinoma cells by upregulating RIOK1, which undergoes phase separation and sequesters IGF2BP1 and G3BP1 into stress granules, suppressing PTEN mRNA translation[61]. How such extrinsic signals converge with intrinsic stress and sublethal death programs to foster DTP emergence remains elusive. Another layer of complexity arises from stochastic transcriptional stress responses. Although it remains untested whether stochastically primed DTP states can licence ISR activation upon drug exposure in cancer, evidence from other systems is compelling. In yeast, Nadal-Ribelles et al. showed that single-cell transcriptional heterogeneity under osmotic stress creates a subpopulation with basal stress-program expression that is hyper-responsive and stress-resistant[62]. Similarly, in chronic viral infection of mammalian cells, Klein et al. demonstrated that ISR activation behaves as a stochastic switch controlled by stress-responsive kinases, generating cell-to-cell variability that shapes adaptation dynamics[63]. Thus, dissecting early stress-response signalling, such as eIF2α phosphorylation and nutrient-sensing kinase activity, in individual cells exposed to SOC therapies will be critical to defining what the drugs leave behind (Fig. 1a).

What the drug imprints: the memory of persistence

DTP cells can repopulate drug-sensitive populations upon treatment withdrawal, a hallmark of their resilience[3]. But are DTP-derived cells truly free of their past? Much like childhood memories shape who we become, transient stresses such as drug exposure or nutrient limitation may imprint lasting "memories" on DTP cells, biasing future fate decisions—whether to divide, persist, or revive.

In the 1940s, Luria and Delbrück demonstrated that bacterial resistance could arise from stochastic events prior to viral infection, laying the foundation for understanding cellular

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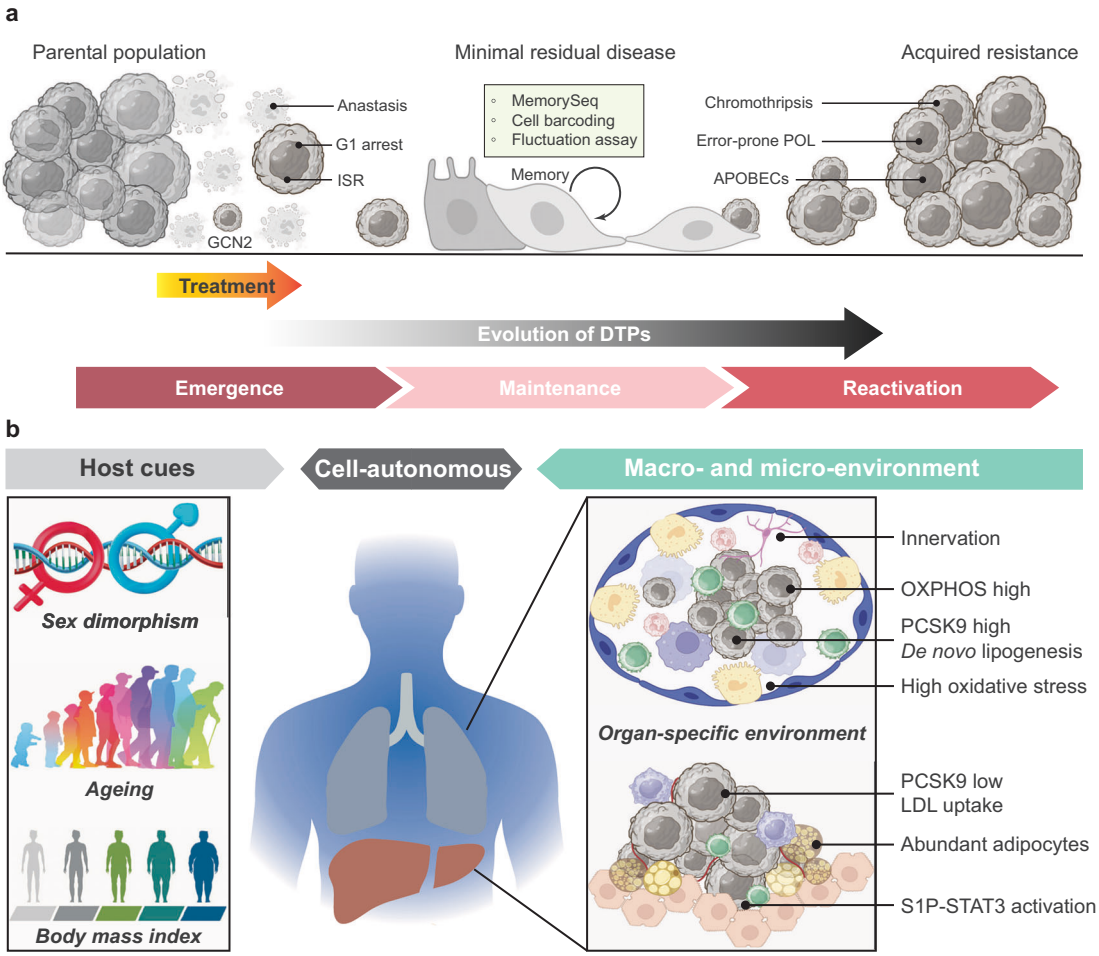

Fig. 1 | The complexity of DTPs. a The evolution of DTP cells upon SOC treatment requires understanding of the immediate responses at the emergence phase, the "memory" maintenance phase and various reactivation mechanisms promoting the genetic acquired resistance. **b** These intrinsic, cell-autonomous mechanisms are intimately associated with the complex micro- and macro-environment of the niches where the DTP cells reside, but also the host cues, ranging from sex, age and metabolic status, that impact the general conditions that may shape the behaviours of DTPs. POL polymerase, ISR integrated stress response, OXPHOS oxidative phosphorylation. Created in BioRender. Shen, S. (2025) https://BioRender.com/xto3poy.

memory and heritability[64]. In mammalian cells, stable transcriptional states may also persist across divisions. Recently, Meir et al. applied a modern Luria–Delbrück framework by growing single colon or lung cancer cells into colonies, then profiling them using single-cell transcriptomic and epigenetic[65]. They uncovered heritable transcriptional modules spanning the epithelial-to-mesenchymal transition (EMT) spectrum, reflecting clonal memory. Similar lineage tracing under drug exposure and withdrawal could illuminate how DTP memory evolves. Shaffer et al. developed MemorySeq (Fig. 1a), a method combining fluctuation analysis with bulk RNA-seq to reveal rare, heritable expression states in lung cancer and melanoma. Memory genes like *EGFR, NGFR* and *AXL* persisted across generations, but gradually decline, suggesting a clock-like loss of memory[66]. Beyond transcriptional regulation, mechanical cues can also imprint lasting effects. For example, breast cancer cells primed on stiff extracellular matrix (ECM) exhibited enhanced collective migration, driven by sustained nuclear localization of YAP, a mechanosensitive transcription factor[67]. YAP knockdown abolished this migratory memory[33,68]. Such mechanical memory may propagate through persistent cytoskeletal remodelling and MAPK signalling, potentially involving RUNX2-dependent transcription that maintains chromatin accessibility at ECM-related genes. In breast cancer models, this memory, acquired in a stiff microenvironment, remains imprinted even after cells disseminate to the softer bone marrow niche[69]. Building on these pioneering studies and given that YAP-driven EMT is a hallmark of multiple DTP models[2], the role of mechanical memory during SOC treatment remains an uncharted direction in DTP research. Another hallmark of DTP cells is metabolic reprogramming. Residual breast cancer cells, for instance, retain elevated glycolysis, altered metabolite levels, and distinct DNA methylation patterns even after oncogenic signals are inhibited[70]. These features reflect a retained imprint of the tumour's metabolic past, maintained through gene expression, metabolism, and epigenetic regulation.

To test whether DTP cells "remember" their past drug exposure, longitudinal tracking of individual cells or clones is needed. Technologies like single-cell sequencing and lineage tracing offer powerful tools to uncover DTP state dynamics. Harmange et al. combined cell barcoding with single-cell RNA-seq in melanoma, identifying TGF-β and PI3K signalling as regulators of memory state switching[7]. Sequential inhibition of PI3K followed by BRAF/MEK targeting, disrupted the primed state and prevented resistance. This highlights the therapeutic potential of targeting memory-associated mechanisms. Cells can retain environmental information over time through cellular memory[71], often mediated by recurring network motifs. Feedback and feedforward loops underlie distinct memory types, enabling desensitization[72] or bistability[73]. Feedforward loops, in particular, can

drive stress granule formation and priming[74], as seen with RIOK1-mediated assemblies[61]. Moving forward, identifying specific network motifs in DTP cells and analysing their dynamics at the single-cell level will be crucial for tracing the origins of these rewired regulatory circuits and elucidating the heterogeneity of drug-response memory among DTP populations[75,76] (Fig. 1a).

When the silence breaks: mechanisms of persistence-to-resistance transition

DTP cells that evade SOC therapies and immune surveillance can evolve into stably resistant, proliferative populations[2] (Fig. 1a). The timing for DTPs to evolve into fixed, acquired resistance remains poorly understood and likely depends on cancer type and treatment regimen[3]. A major research frontier is deciphering the mechanisms that drive DTP evolution toward resistance.

By definition, this transition requires continued exposure to therapy, under which DTPs can follow diverse evolutionary trajectories[2,17]. For instance, in EGFR-mutant lung adenocarcinoma, targeted therapy induces APOBEC3A-mediated mutagenesis[77] and activates GAS6–AXL signalling[78], driving error-prone DNA repair and nucleotide imbalance that accelerate resistance. In microsatellite instability-high colorectal cancer, EGFR- or BRAF-targeted therapy suppresses mismatch repair, increasing reliance on error-prone polymerases and promoting adaptive evolution[11]. Beyond genetic routes, DTPs can also undergo nongenetic evolution, as shown in lung cancer and melanoma models[17,79]. In addition, an overlooked step in DTP evolution is the proliferative intermediate of drug-tolerant expanded persisters (DTEPs), first described by Sharma et al.[5]. These cells re-enter the cell cycle under continued therapy yet remain reversible, providing a staging ground for stable resistance. Both quiescent DTPs and slow-cycling persisters can give rise to DTEPs, as shown in BRAF-mutant melanoma, where subsets rapidly escape quiescence via ERK and mTORC1 converging on cyclin D1 signalling[80]. Capturing this transient cycling stage will be key to understanding relapse, and emerging tools like cell barcoding, single-cell trajectories, and live-cycle reporters will offer the means to dissect dormancy exit versus slow-cycling heterogeneity.

There is also growing recognition that replication stress–driven genomic instability plays a critical role in facilitating tumour cell escape from cell cycle arrest[81]. Whole-genome sequencing of methotrexate-resistant HeLa cells and BRAFV600E-mutant colorectal cancer biopsies resistant to vemurafenib shows that extrachromosomal DNA (ecDNA) amplification, often triggered by chromothripsis, fuels rapid oncogene amplification and accelerates therapy resistance[82]. Shoshani et al. showed that this process is mediated in part by poly(ADP-ribose) polymerase (PARP) and DNA-dependent protein kinase (DNA-PK), which help tether ecDNAs to chromosomal ends under drug-induced DNA damage[82]. In other solid tumours like melanoma, DNA-PK–mediated rearrangements promote amplification of resistance-driving nonhomologous end joining genes via ecDNA, contributing to extensive chromothriptic regions in both PDX and patient tumours resistant to MAPK-targeted therapy[83]. In SCLC, serial patient-derived xenograft models have demonstrated that cross-resistance to multiple therapies can arise through MYC amplification on ecDNA, a phenomenon recurrently observed in clinical samples of cross-resistant SCLC[84]. Using CRISPR-Cas9 screening, Engel et al. uncovered a non-canonical role for the Fanconi anemia (FA) DNA repair pathway in promoting chromothripsis[85]. Specifically, mono-ubiquitination of the FANCI–FANCD2 complex facilitates its recruitment to chromosomes enclosed in micronuclei. This, in turn, activates error-prone DNA synthesis mediated by the polymerase POLD3, promoting aberrant reassembly of fragmented chromosomes[85]. Paradoxically, this pathological engagement of the FA repair machinery contributes to genomic instability and clonal evolution through chromothripsis (Fig. 1a).

Although these studies were not explicitly conducted in the context of DTPs, ecDNA-associated genomic instability may represent an emerging coherent mechanism driving the evolution of DTPs into acquired resistance across diverse treatment and cancer types[86]. Indeed, micronuclei formation is frequently observed in cells surviving therapy-induced apoptosis and undergoing anastasis[87]. Intriguingly, hypermutability does not always drive therapeutic escape. In colorectal cancer, treatment with cisplatin and temozolomide induces mismatch repair inactivation, leading to hypermutation and neoantigen generation, which triggers immune-mediated tumour clearance[88]. Thus, a deeper understanding of DTP biology will require integrative approaches that consider both tumour-intrinsic and host-related factors.

Moving forward beyond isolation toward integration

Despite advances in DTP biology, how host systems shape DTP cells behaviour remains poorly understood. As cancer research moves toward addressing systemic and environmental influences[14], it is increasingly clear that residual disease does not persist in isolation. Future efforts must integrate these complexities to fully understand and effectively target DTP cells in a clinically relevant manner.

The choreography of DTP cells within the microenvironment

Research on DTP cells rely heavily on in vitro models such as established cell lines or PDOs, primarily focusing on cell-intrinsic survival mechanisms. However, emerging evidence highlights the critical role of the tumour microenvironment in shaping the DTP state. A recent in vitro screen demonstrated that environmental factors—including fibroblast growth factor 2, hepatocyte growth factor (HGF), insulin-like growth factor 1, and interferon-γ (IFNγ)—can promote targeted therapy tolerance in lung cancer and melanoma cells[89].

While classically anti-tumorigenic, type I interferons induced after immunogenic chemotherapy have been shown to reprogram tumour cells into a CSC-like state across diver cancer types[90]. This effect is mediated in part by the interferon-stimulated gene KDM1B, which epigenetically activates gene programs linked to stemness, EMT, and tissue regeneration. Type I interferons can also induce senescence via CDKN2A or CDKN1A. In RIP-Tag2 pancreatic tumours, deletion of $p16^{Ink4a}/p19^{Arf}$ (Cdkn2a) or $p21^{Cip1}$ (Cdkn1a) promotes immune evasion and resistance to anti-PD1 immune checkpoint blockade (ICB), mirroring rapid progression in metastatic melanoma patients under ICB[91]. Beyond interferons, IL-17A secreted by $CD4^+$ T cells has been shown to awaken metastatic dormant 4T07 breast cancer cells in a mouse model[92]. He et al. used recombinase-based tracing in a metastatic breast cancer model with lung dissemination, showing that chemotherapy-induced senescence in lung fibroblasts drives secretion of CXCL1, MIF, and complement C3, promoting neutrophil extracellular trap (NET) formation[93]. These NETs remodel the ECM and trigger reactivation of dormant disseminated breast cancer cells[93]. Although these studies were not conducted in the DTP context, they provide compelling evidence that similar microenvironment-mediated mechanisms may regulate DTP cell survival, dormancy, and reactivation, warranting further investigation.

On the other hand, DTP cells are not merely passive survivors; they can actively shape their microenvironment through immunomodulatory mechanisms. In lung adenocarcinoma, for example, DTP cells that emerge following EGFR inhibitor treatment (e.g., osimertinib) show epigenetic upregulation of the immunosuppressive receptor CD70, which facilitates immune evasion by engaging CD27 on T cells[30]. Similarly, in *TP53* wild-type breast cancers, residual tumour cells following chemotherapy exhibit transcriptionally distinct subpopulations: one enriched in IRF1-driven PD-L1 expression, and another in CD80 expression regulated by p53 signalling[94]. These findings highlight the

heterogeneity of DTP cells and the diverse mechanisms they employ to escape immune surveillance. Beyond expressing immunoregulatory proteins, cancer cells can directly impair infiltrating T cells through intercellular transfer of dysfunctional mitochondria containing mutated mtDNA[95], a possible trait to be explored in DTP context. DTP cells may also benefit from cancer cell cooperative interactions within the tumour. In colorectal cancer, chemotherapy-induced tumour cell death leads to ATP release, which activates a survival program in neighbouring tumour cells through the purinergic receptor P2X4 and mTOR signalling, promoting their transition into a DTP state[96]. These examples reveal a multifaceted and dynamic role of DTP cells in modulating both immune and tumour cell networks to ensure survival. However, these mechanisms likely represent only a fraction of DTP complexity. Future work should explore how other cytokines and stromal signals, such as TGF-β[97], HGF[98] and MIF[99], reprogram DTP cell states. Crucially, studies must also account for the heterogeneous cell populations in the residual tumour microenvironment to fully elucidate the choreography of DTP cells in clinically relevant settings.

The tapestry of the macroenvironment

In clinical oncology, systemic therapies are widely used for advanced cancers, yet tumour location can significantly impact treatment response. For example, liver metastases reduce the efficacy of ICB by attracting and eliminating $CD8^+$ T cells via $FasL^+$ macrophages[100]. This highlights how organ-specific macroenvironments modulate therapy outcomes. It remains unknown whether DTP cells in different organs adopt distinct features or therapeutic sensitivities, but studies on cancer organ tropism may offer useful parallels to explore this emerging area.

In pancreatic ductal adenocarcinoma (PDAC), metastatic cells colonizing the lung or liver display different phenotypes and dependencies. Lung metastases exhibit a well-differentiated histology and express classical subtype markers such as GATA6, whereas liver metastases are poorly differentiated and express mesenchymal markers like ZEB1[101]. Rademaker et al. identified the cholesterol metabolism regulator PCSK9 as a key determinant of these organ-specific phenotypes. PDAC cells with low PCSK9 expression preferentially colonize the liver, where they exploit the local abundance of low-density lipoprotein (LDL) by enhancing LDL uptake and activating mTORC1 signalling through cholesterol-derived metabolites. In contrast, PCSK9-high PDAC cells metastasize to the lung, upregulating de novo cholesterol biosynthesis[101]. This endogenous lipid production yields intermediates such as 7-dehydrocholesterol, which protect tumour cells from ferroptosis, an oxidative form of cell death exacerbated in the oxygen-rich lung environment. Similar metabolic adaptation is observed in melanoma. Tumour cells scavenge phosphatidylcholines from subcutaneous adipocytes, boosting PI3K-AKT signalling, fatty acid oxidation, and oxidative phosphorylation (OXPHOS)[102]. High-OXPHOS melanoma cells preferentially metastasize to the lung and brain, while reducing oxidative stress redirects them to the liver, where a ceramide-induced S1P-STAT3-IL6 signalling axis promotes liver tropism[102]. These findings underscore how organ-specific macroenvironmental features, particularly lipid availability and oxidative stress, shape tumour cell states. This has implications for DTP biology, as DTPs are especially vulnerable to ferroptosis[103], suggesting that their survival and evolution may vary by organ (Fig. 1b). In addition to metabolic influences, organ-specific immune environments also affect tumour phenotypes. In the leptomeninges, IFNγ-driven innate immunity suppresses metastases independent of adaptive immune responses[104]. Remsik et al. found that leptomeningeal T cells produce IFNγ, recruiting peripheral myeloid cells that differentiate into dendritic cell subsets, including $CCR7^+$ populations that activate natural killer (NK) cells[104]. These findings highlight how organ-specific immune landscapes can significantly alter tumour-immune dynamics, with potential relevance to DTP cell persistence and immune evasion.

From a clinical standpoint, such organ-level heterogeneity may help explain the phenomenon of dissociated responses, wherein different lesions within the same patient exhibit variable sensitivity to therapy[105]. While the prognostic implications of such responses remain uncertain and appear to vary by treatment and cancer type, they strongly suggest that organ-specific factors influence tumour cell state and therapy sensitivity at the time of treatment. The existence of heterogeneous DTP populations across organs may be a contributing factor. The field of DTP would benefit from incorporating organ-specific analyses, including spatial transcriptomics, metabolic profiling, immune landscape mapping and tumour-nerve system interactions[106]. Although this adds another layer of complexity, such efforts are essential to fully reveal the behaviour of DTP cells in real-world clinical settings.

The complex cues of the host

Cancer progression and treatment response are not only shaped by tumour-intrinsic factors but also by host physiology, including sex, age and metabolic status (Fig. 1b). While reductionist cancer models provide insights into key features of DTP cells, they fall short in capturing the complex, systemic influences of the host.

Age, for instance, is a well-established risk factor for cancer and is accompanied by immunosenescence[107]. Yet, clinical trials suggest that older patients often respond as well or even better to ICB than younger individuals[108,109]. This may reflect age-related shifts in tumour molecular landscapes driven by differential selection pressures[110], including increased mutational burden, heightened immune checkpoint gene expression, and enhanced IFNγ signalling[111], all of which may influence both therapy response and DTP states. At the tissue level, ageing alters the tumour microenvironment. In aged mouse lungs, S1PR1-mediated expansion of γδT17 cells promotes a premetastatic niche by recruiting KIT^+CXCR4^+ neutrophils that suppress $CD8^+$ T cells[112]. Concurrently, aged lungs support reactivation of dormant melanoma cells, driven by age-induced fibroblast remodelling that increases sFRP1 secretion, suppressing WNT5A and activating AXL/MER pathways to promote proliferation[113]. These findings highlight the need to study DTPs in age-appropriate models, as most preclinical studies use young mice (6–8 weeks), whereas 18-month-old mice better reflect the geriatric human condition in which most cancers occur.

Sex and age are closely intertwined in shaping immune responses (Fig. 1b). In females, immune function is influenced by hormonal fluctuations across the menstrual cycle, pregnancy and menopause. In males, age-related declines in testosterone levels also alter immune activity[114]. These differences contribute to sex-specific variations in anti-tumour immunity and treatment outcomes, making sex an important factor in studying DTP cells. A large retrospective analysis by Litchfield et al. found that male sex correlated with stronger responses to ICB across cancer types, independent of tumour mutational burden, T cell infiltration, or tumour genetics[115]. These effects may arise from hormone-mediated modulation of the tumour microenvironment. For instance, in castration-resistant prostate cancer, androgen receptor (AR) expression on $CD8^+$ T cells suppresses TNF, granzyme B, and IFNγ, impairing cytotoxicity[116]. Similarly, AR signalling limits T cell function in bladder cancer by downregulating TCF1[117]. In contrast, estrogen receptor (ER) activity in melanoma can polarize macrophages toward immunosuppressive phenotypes, reducing efficacy of immunotherapy in females[118]. Beyond hormones, sex chromosomes may also contribute. Loss of *XIST*, which silences one X chromosome in females, enhances ovarian cancer cell stemness and plasticity[119]. These findings raise important questions about whether DTP states should be defined in a sex-specific manner.

Hormonal and age-related factors are also closely linked to individual metabolic status (Fig. 1b). In breast cancer, postmenopausal women with obesity face over a 50% higher risk of developing the disease, reflecting the well-established pro-tumorigenic role of obesity. Paradoxically, higher body mass index (BMI) has been associated with improved treatment outcomes in some cancers[120]. A large retrospective study in melanoma found that patients with obesity had better progression-free and overall survival than those with normal BMI, particularly among males receiving targeted or immune therapies[121]. These observations highlight the complex interplay among sex, metabolism, and therapy response. At the cellular level, obesity may influence cancer cell plasticity. In a genetically engineered mouse model of breast cancer, obesity increased the number of residual tumour cells following oncogene withdrawal[122]. However, whether these cells represent distinct DTP cell states remains unclear. Additionally, the obesity-related host environment can promote reactivation of dormant tumour cells by enhancing vascular invasion, likely through a systemic proangiogenic and inflammatory milieu enriched in lipocalin-2, VEGF and FGF[123]. These findings further underscore the need to move beyond studying DTPs in isolation. A more integrated understanding of DTP biology, encompassing intrinsic tumour features, local microenvironments, and systemic host status, will be essential to effectively target these therapy-resilient cells.

Future strategies to leverage DTP complexities

Advancing the study of DTPs in more systemic and clinically relevant settings does not mean abandoning traditional cell culture models. In fact, many pivotal discoveries over the past decade have emerged from simple monolayer culture systems. While these reductionist models lack the complexity of in vivo environments and the influence of systemic host factors, with their scalability and controllable perturbations, in vitro models remain highly valuable for dissecting tumour-intrinsic mechanisms of persistence. For example, Pfeifer et al. performed genome-wide CRISPR screening in EGFR-mutant lung cancer to map pathways of persistence to osimertinib or gefitinib, identifying the Hippo/YAP pathway as a key non-genetic survival mechanism under osimertinib[124]. Chen et al. integrated an engineered suicide switch with high-throughput genetic and drug screens in EGFR-mutant PC9 cells, uncovering the BET pathway as a selective vulnerability of lung cancer DTPs[6]. In vitro models can also be extended to 3D cultures and tumour organoids, which have revealed diapause-like persistent states in colorectal cancer[36]. In addition, incorporating microenvironmental factors, such as co-culture with immune or stromal cells or physiological cytokine dosing[89], further enhances the clinical relevance. Using lung cancer patient-derived co-cultures, Hu et al. identified three CAF subtypes, defined by intrinsic TGFβ signalling, that shape distinct therapeutic paradigms[125]. Similarly, a melanoma-cytotoxic T lymphocyte (CTL) co-culture system uncovered antigenic persister cells that exploit sublethal caspase activity to evade IFNγ/Granzyme B-mediated CTL killing[126]. Extending studies to cell–cell interactions could also advance DTP-targeting therapeutics in a transformative way. For example, in EGFR-mutant NSCLC, TROP2 was found enriched across multiple cell lines and PDX models, enabling the development of a Sacituzumab-based, TROP2-directed CAR-T therapy targeting DTP cells[127]. These pioneering studies highlight the strength of in vitro models in uncovering causal mechanisms and cell-cell interactions that are difficult to resolve in patient samples, given their scarcity and heterogeneity. Moving forward, pairing such in vitro mechanistic screens with on-treatment biopsies or minimal residual disease samples will be key to bridging the gap between bench and bedside in DTP research (Fig. 2).

Following this concept, it will be valuable to combine on-treatment single-cell analysis with longitudinal sampling in both preclinical and clinical models. For example, in $KRAS^{G12C}$-mutant lung cancer cell lines, early adaptive responses to KRAS inhibitors were observed, with cells producing active, drug-insensitive $KRAS^{G12C}$ and resuming proliferation shortly after treatment[128]. This strategy can be further enhanced by integrating cell barcoding-based lineage tracing. Cotton et al. employed such a system coupled with longitudinal single-cell RNA-seq to map the origins and clonal evolution of DTPs at high resolution, revealing that cells with elevated baseline expression of survival pathways, including YAP1 and EMT, adapt their transcriptional programs in response to EGFR-targeted therapy in NSCLC[129]. By using cell barcoding in acute myeloid leukaemia, a study showed that minimal residual disease gives rise to low- and high-output tumour clones with stem-like transcriptional programs, where clonal dominance was maintained intrinsically through antigen presentation suppression and SLPI secretion[130]. Although these strategies reveal transcriptional programs at the phenotypic level, an important additional layer is the role of post-translational modifications (PTMs) in DTP biology. Whether PTMs or their regulating enzymes drive DTP formation or survival remains largely unexplored. PTMs orchestrate key signalling and cellular processes across cancers, and analysis of 1110 tumours spanning 11 types reveals conserved acetylation and phosphorylation patterns controlling DNA repair, metabolism, immune response, kinase specificity, and chromatin dynamics[131], all intimately linked to stress-adaptive programs in DTPs. High-resolution PTM profiling could uncover the adaptive signalling switches that allow persisters to survive therapy and evolve resistance, complementing transcriptional and epigenetic studies and providing rationales for druggable targets and novel combination therapies (Fig. 2).

Given the heterogeneity of DTPs, it will be intriguing in the DTP field to identify both common and cancer-specific features of DTP cells across tumour types and treatments. A recent study tackled this question using multi-omics data from over 80 datasets across 127 patient-derived xenograft models of triple-negative breast cancer[132]. Despite treatment heterogeneity, they uncovered shared hallmarks of persister states, including basal keratin expression, activation of stress-response and inflammatory pathways, and a core regulatory network involving AP-1, NFκB, and IRFs[132]. Although limited to one cancer type, this framework could be extended to pan-cancer analyses. Advances in integrative bioinformatics now allow the combination of bulk and single-cell datasets to systematically map the molecular programs underlying cancer plasticity[133]. Several robust data integration tools support cross-tumour analyses at increasing scale and resolution[134]. For example, Tagliazucchi et al. reconstructed EMT trajectories from transcriptomic data across more than 7000 tumours, revealing three distinct macro-states with prognostic and therapeutic relevance[135]. Spatial transcriptomic approaches have similarly uncovered local environmental cues driving EMT in breast cancer[136].

Moreover, integrating large-scale genomics, transcriptomics, and functional screens[137] can identify non-canonical transcriptional modules[138] and reveal shared or context-specific vulnerabilities in DTP programs across cancer types[124] (Fig. 2). With the rapid advancement of artificial intelligence (AI), integrating AI into large-scale data analyses, for predicting treatment response and relapse[139], discovering PTMs[140], novel target discovery[141] and designing combination therapies[142] from perturbation experiments, will likely transform DTP research. Notably, recent developments in large language models (LLMs) have enabled "virtual labs", where an LLM principal investigator coordinates specialist LLM agents to design complex experiments, such as virus-targeting nanobodies[143]. This agent-based architecture could be potentially adapted to DTP biology, enabling AI-human collaboration to tackle fundamental questions in tumour persistence and resistance.

While clinical persistence has been discussed extensively in recent reviews by us and others[3,144], this perspective also emphasizes the importance of using suitable animal models to investigate how host factors and the macroenvironment influence DTP evolution. For example, to study the impact of metabolic status, both diet-induced obesity and genetically engineered obese mouse models can be used

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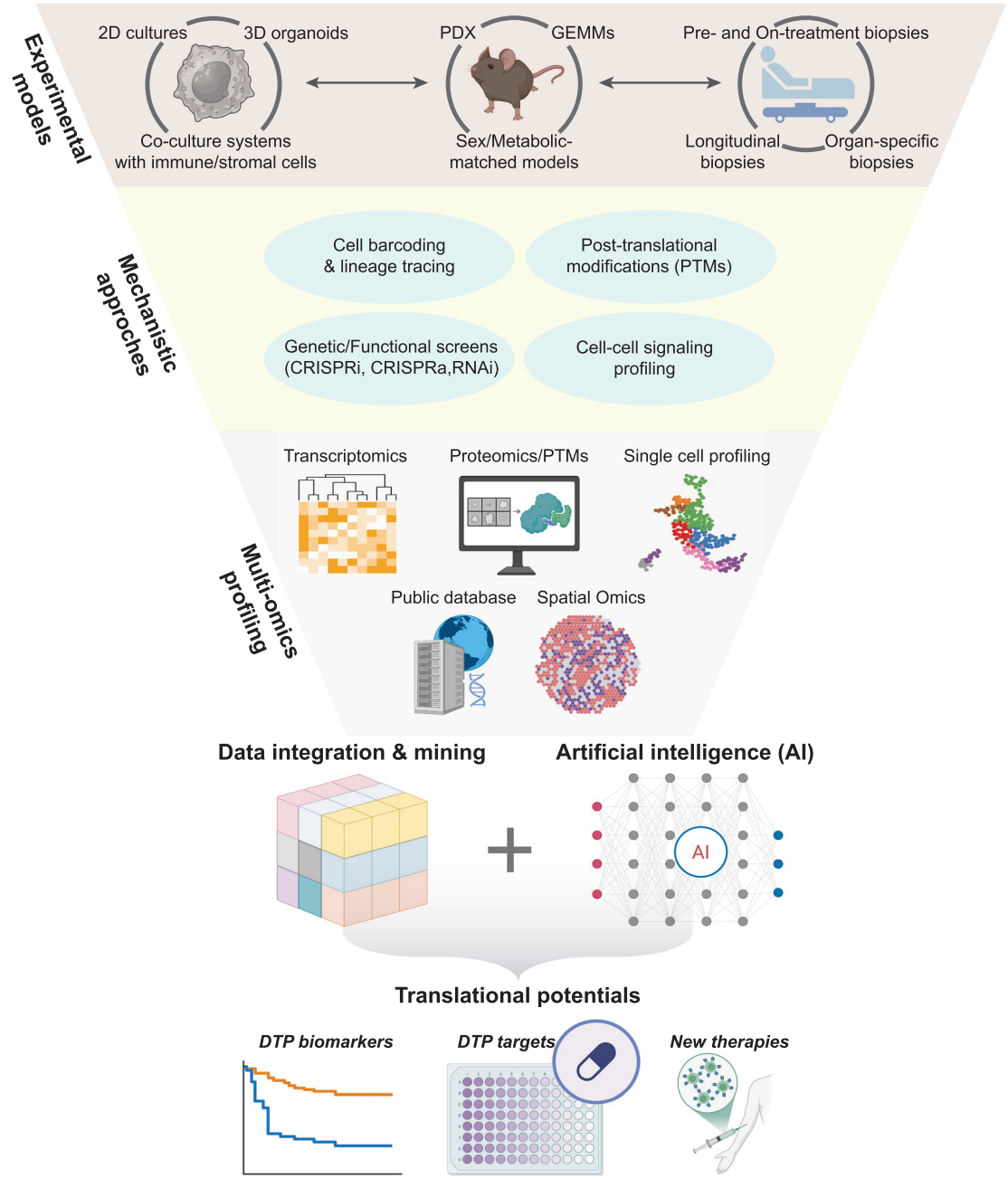

Fig. 2 | Future technologies to leverage DTP complexity. By leveraging a spectrum of DTP models—from 2D monolayer cultures and 3D organoids to co-culture systems, context-dependent mouse models and clinical samples—combined with innovative technologies such as multi-omics profiling, perturbation screens, computational integration and AI-driven discovery, researchers can begin to unravel the complexity of these resilient cells and translate insights into clinically relevant biomarkers, novel targets, and therapeutic strategies against DTPs. Created in BioRender. Shen, S. (2025) https://BioRender.com/ayky3zw.

to mimic relevant host conditions[145]. Comparing residual tumours from lean and obese hosts may uncover distinct DTP programs shaped by metabolic context, as host metabolic status has been implicated in therapy resistance in pancreatic cancer[146] and in modulating tumour-associated immune responses more broadly[147]. In this light, expanding the use of clinically relevant experimental models will be essential for capturing the full complexity of DTP biology and the dynamic evolution of residual disease across diverse host environments.

Conclusions

Fulfilling the bench-to-bedside gap in DTP research will require a truly integrated approach that combines mechanistic insights from reductionist models with the complexity of in vivo systems and clinical contexts. By aligning innovative in vitro strategies with high-resolution single-cell profiling, robust computational tools, clinically relevant models, and transformative AI-based approaches, the field is now well-positioned to uncover the molecular logic of tumour persistence. Collaborative efforts to expand biobanks, optimize sampling strategies, and model diverse host conditions will be essential. Ultimately, translating these insights into predictive biomarkers and therapeutic strategies will be key to overcoming residual disease and improving patient outcomes.

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Author contributions

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