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Review

Stochastic Population Dynamics of Cancer Stemness and Adaptive Response to Therapies

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Abstract

Intratumoral heterogeneity can exist along multiple axes: Cancer Stem Cells (CSCs)/non-CSCs, drug-sensitive/drug-tolerant states and a spectrum of epithelial-hybrid-mesenchymal phenotypes. Further, these diverse cell-states can switch reversibly among one another, thereby posing a major challenge to therapeutic efficacy. Therefore, understanding the origins of phenotypic plasticity and heterogeneity remains an active area of investigation. While genomic components (mutations, chromosomal instability) driving heterogeneity have been well-studied, recent reports highlight the role of non-genetic mechanisms in enabling both phenotypic plasticity and heterogeneity. Here, we discuss various processes underlying phenotypic plasticity such as stochastic gene expression, chromatin reprogramming, asymmetric cell division and the presence of multiple "attractors". These processes can facilitate a dynamically evolving cell population such that a subpopulation of (drug-tolerant) cells can survive lethal drug exposure and recapitulate population heterogeneity on drug withdrawal, leading to relapse. These drug-tolerant cells can be both pre-existing and also induced by the drug itself through cell-state reprogramming. The dynamics of cell-state transitions both in absence and presence of the drug can be quantified through mathematical models. Such a dynamical systems approach to elucidating patterns of intratumoral heterogeneity by integrating longitudinal experimental data with mathematical models can help design effective combinatorial and/or sequential therapies for better clinical outcomes.

Keywords: Cell-state transitions; Phenotypic plasticity; Cancer Stem Cells; Intratumoral heterogeneity; Lamarckian Induction; Drug resistance

Introduction

Intratumor heterogeneity has emerged as an Achilles' heel in cancer treatment. It is a multifaceted phenomenon with inputs from genetic, epigenetic and other environmental axes, including the selection pressure applied by various therapeutic assaults (1). Most attempts to longitudinally track this heterogeneity have been from a clonal evolution perspective or developmental hierarchy of tumor-initiating cells, by mapping the corresponding mutational profiles (2). However, increasing evidence indicates that cancer progression can also evolve in a mutation-independent manner (3). These non-genetic changes can drive cancer cell adaptation to various stresses such as therapy, hypoxia *etc.* and enable cells to reversibly and rapidly change their phenotypes during metastasis (4). Thus, elucidating population dynamics of phenotypic plasticity and non-genetic (phenotypic) heterogeneity is crucial to understand and eventually target cancer cell survival and adaptation.

Phenotypic plasticity and heterogeneity has been well-investigated in microbial cell populations surviving in dynamic environments (5), and is being identified extensively in cancer cell populations in the past 15 years (6–10). The sources for cell-to-cell heterogeneity can include, but not limited to, stochastic gene expression (11), asymmetric cell division (12), cell cycle phase (7), and epigenetic alterations (8) (**Fig 1**). These sources contribute to intercellular differences in protein abundance that can lead to heterogeneous cellular response to a uniform environment (6).

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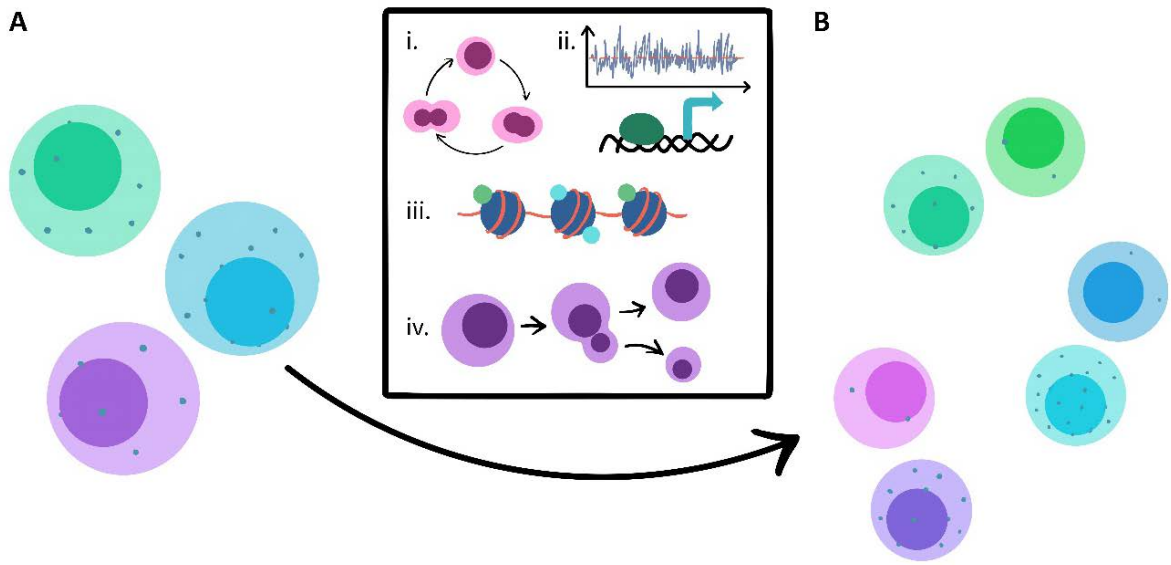

Fig 1. Sources of non-genetic heterogeneity*. Cells with distinct colours (green, blue, and purple) represents genetic variants (**A**) and non-genetic heterogeneity shown by shades of the same colour (**B**). The inset depicts schematic for sources of cell-to-cell variability – **i.** Differences in cell-cycle phase among cells, **ii.** Stochastic gene expression, **iii.** Epigenetic differences (at histone or DNA level) among cells, and **iv.** Asymmetric cell division – asymmetric distribution of molecular content to daughter cells.*

Besides the abovementioned factors, phenotypic heterogeneity can also emerge from the complex dynamics of interactions among many bio-molecules (proteins, microRNAs, epigenetic factors, metabolites *etc*.) that allow for (co-)existence of multiple “attractors”, i.e. specific molecular patterns that correspond to different cell phenotypes. Under specific perturbations, including those driven by stochastic intracellular fluctuations, cells can switch from one attractor to another reversibly, thereby achieving phenotypic plasticity (13). Such plasticity can promote bet-hedging – an evolutionary strategy through which an isogenic population can maximize its fitness in dynamic environments by giving rise to many subpopulations (phenotypic heterogeneity) (14). This strategy is well-illustrated through ‘drug-tolerant persisters’ (DTPs) – a subpopulation of cancer cells that survive drug treatment without *de novo* genetic mutations but by manifesting a slow-growth phenotype in the presence of drug and resuming the initial behavior upon drug removal (15). DTPs have been observed across cancer types – breast cancer, liver cancer, melanoma, lung cancer, prostate cancer – in response to chemotherapy and targeted therapy, and remains an insuperable obstacle to durable anticancer treatment (15).

Besides drug-induced transitions exemplified by DTPs, spontaneous transitions among many cell phenotypes have been observed. For instance, Cancer Stem Cells (CSCs) – a subpopulation of tumor cells that can self-renew and differentiate into other phenotypes – were earlier thought to seated at the apex in the hierarchy of cellular differentiation (16). However, recent evidence across cancer types has shown that a) non-CSCs can acquire the traits of CSCs stochastically (17,18), and b) CSCs include many subpopulations that may reversibly switch among one another (19,20). The transition rates among different cellular phenotypes can, of course, be influenced by external conditions such as drug treatment (20,21). Thus, combinatorial and/or sequential strategies that can overcome the adaptive strategies of cancer cell subpopulations by specifically targeting the vulnerabilities of a drug-induced transition are gaining increasing attention (22,23). For instance, exposure to taxanes induces a phenotypic transition to $CD24^{hi}$ $CD44^{hi}$ chemotherapy-tolerant state with activated Src Family Kinase (SFK). Thus, pharmacological inhibitors of SFK, given after the treatment with taxanes but not by co-administration, can improve the antitumor drug outcome (23).

Here, we review various reports on population dynamics of stochastic phenotypic switching and drug-induced transitions in multiple cancers, while drawing parallels with developmental contexts. Finally, we discuss how

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mathematical modelling at intracellular and population dynamics levels have helped in decoding these transitions and make further experimentally testable predictions.

Spontaneous state transition in development and cancer

An iconic metaphorical representation of phenotypic plasticity, particularly in context of embryonic development, is given by Waddington's landscape. A ball that rolls down this landscape made of branching valleys denotes the differentiation trajectory of a cell (24). This diagram conveys the idea of lineage segregation and commitment towards specific fates, as the ball crosses successive branching or bifurcation points. But cellular identities assigned "at birth" during development can change during the lifespan of an organism, through de-differentiation (conversion of a mature cell into a progenitor-like state) or trans-differentiation (conversion of one mature cell type to another) (24). These mechanisms constitute normal injury response and are important for regeneration (25).

However, phenotypic plasticity is not restricted to being an injury response or an outcome of cellular reprogramming cocktails alone; it can be seen spontaneously as well. Such spontaneous cell-state transitions can maintain a dynamic equilibrium in a population (**Fig 2A**). For instance, when the three subpopulations of clonal hematopoietic progenitor cells with varying Sca-1 levels were segregated and cultured separately, they reconstituted the parental distribution after a week, indicating cell-state transitions among the Sca-1^{low}, Sca-1^{medium} and Sca-1^{high} fractions (26). Similar observations showing return towards equilibrium proportions were reported in CD44^{hi} CD24^{neg} EpCAM^{lo} (stem-like), CD44^{hi} CD24^{neg} EpCAM^{neg} (basal) and CD44^{lo} CD24^{hi} EpCAM^{hi} (luminal) subpopulations in SUM149 and SUM159 breast cancer cells (17) (**Fig 2B**). The relative abundance of these subpopulations can be altered by treatment with drugs; for instance, paclitaxel increased the frequency of luminal state (17). In another case, EpCAM^{hi} (epithelial) and EpCAM^{lo} (mesenchymal) subpopulations in PMC42-LA breast cancer cells returned to a 80:20 parental distribution, after being segregated (27). However, the return towards parental distribution is not always observed. For instance, in prostate cancer PKV cells, when the subpopulations – epithelial (E), mesenchymal (M) and hybrid E/M – were sorted and cultured independently, they all switched among one another but gave rise to different population distributions after two weeks, depending on initial conditions. While individual E and M subpopulations switched to a 80% E and 80% M population distribution respectively, only 20% hybrid E/M cells maintained their phenotype (28). Further, in MCF7 and SUM149 breast cancer cells, two subpopulations of CSCs (CD44^{hi} CD24^{lo} – mesenchymal, ALDH1^{hi} – hybrid E/M) were reported in specific ratios. When sorted and cultured for 10 days, they did interconvert among one another and gave rise to non-CSCs as well, but did not recapitulate the parental distribution ratio at the mentioned timepoint (19). These examples highlight a dire need to quantify population dynamics at multiple and extended time points to infer the underlying principles of population dynamics and plasticity.

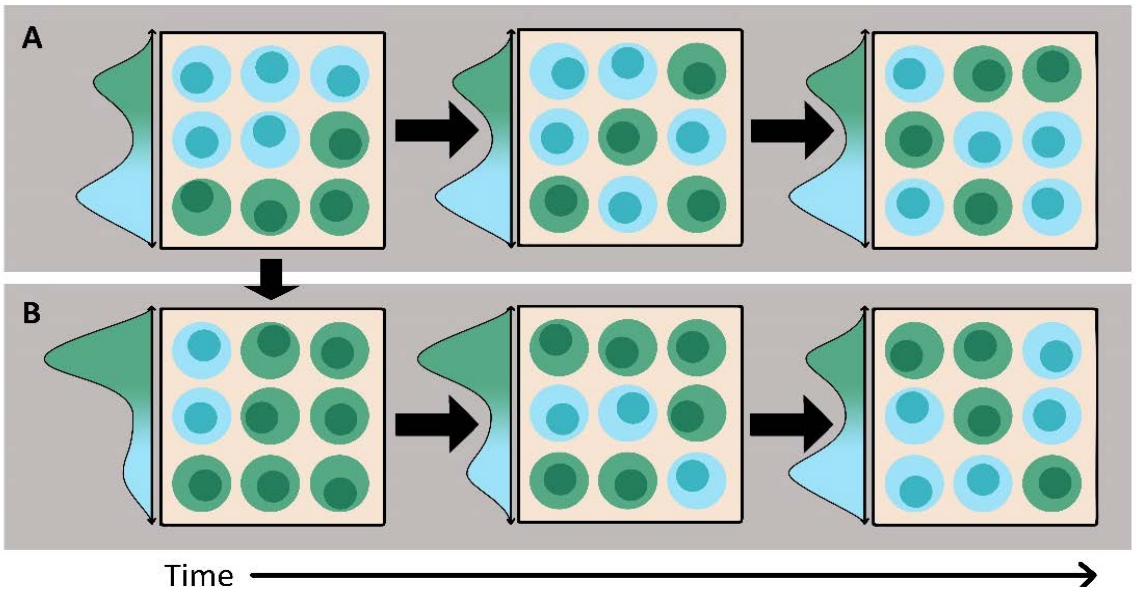

***Fig. 2 Spontaneous cell-state transition shaping phenotypic heterogeneity**. **A)** A population of cells, inside square boxes, that maintain a fixed ratio between two distinct phenotypes (coloured green and blue) over time, despite stochastic cell-state transitions, showcasing a dynamic equilibrium. Histogram on the left of each square box represents*

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the composition of the population. ***B)*** *Regaining of original population distribution of phenotypes upon a perturbation (drug treatment, subpopulation segregation etc.). On enriching green phenotype in a population which is otherwise blue phenotype dominated (left plots in panels A, B), the population regains blue phenotype dominance over time (B).*

Phenotypic plasticity is not restricted to *in vitro* observations, but also vividly seen *in vivo*. Luminal and basal breast cancer SUM159 subpopulations were observed to regenerate functional stem-like cells *in vivo* (17). Similarly, among the multiple single-cell clones established from SUM149, tumors formed by hybrid E/M clones were the most heterogeneous ones (29), reminiscent of higher plasticity seen for hybrid E/M phenotypes as compared to the extreme E and M ones *in vitro* (28). Consistently, in squamous cell carcinoma, subcutaneous transplantation of tumor subpopulations – each with a varied epithelial-mesenchymal status – led to a distinct proportion of each subpopulation in tumors at both early (3-4 weeks) and late (7-8 weeks) time points (30).

Multiple factors can influence these transition rates from one phenotype to another. First, relative depth of different "attractors" – each corresponding to a specific phenotype – can govern this dynamics. The deeper an attractor (or a Waddington landscape's valley), the more difficult it gets to transition out from that phenotype. Multiple mathematical attempts to construct such landscapes for intracellular transcriptional networks driving E-M plasticity have shown that the "attractors" corresponding to hybrid E/M states are relatively shallow as compared to those corresponding to E and M states (31,32). This salient feature of hybrid E/M cells can help explain their higher plasticity (28–30). Second, underlying chromatin marks can influence the depth of an "attractor" in a landscape, thus contributing to "resistance" to a state-transition. For instance, upregulating miR-200 levels drove a Mesenchymal-Epithelial Transition (MET) in MDA-MB-231 breast cancer cells, but failed to do so in RD sarcoma cells, unless the chromatin remodelling protein BRG1 was inhibited (33). Third, transition rates can depend on the previous "history" of a cellular population. For instance, when MCF10A cells were pushed to undergo Epithelial Mesenchymal Transition (EMT) through induction with TGFβ for short durations (3-6 days), most of them returned to their initial phenotypes upon TGFβ withdrawal. But, upon long term treatment (9-12 days), reversibility was significantly constrained (34). The authors, using a phenomenological model, postulated that time-dependent epigenetic changes caused the lack of reversibility (34). However, longitudinal ChIP-Seq and/or ATAC-seq data, concurrent with RNA-seq data, would be important to investigate this hypothesis further. Fourth, besides intra-cellular dynamics, growth media composition can alter transition rates among the phenotypes (35). For instance, re-equilibration dynamics of phenotypic heterogeneity in cancer cells is affected by choice of growth medium, as seen for $CD24^{neg}$ (non-stem-like) and $CD24^{pos}$ (stem-like) phenotypes in basal breast cancer MCF10CA1a cells (21).

Overall, these observations illustrate that spontaneous phenotypic plasticity and heterogeneity can perpetually exist in both normal and cancer cell populations, and is governed by many intertwined stochastic regulatory mechanisms.

Drug-induced state-transitions (Lamarckian induction): hallmarks of phenotypic plasticity

Phenotypic plasticity and heterogeneity can help cancer cells evade multiple therapies, leading to a significant bottleneck in cancer treatment (**Fig 3A-B**). The ubiquitous presence of cell-to-cell heterogeneity in protein concentrations in many pro- and anti-apoptotic cascade molecules can allow for one or more subpopulations in isogenic cells to survive drug insults, a phenomenon known as fractional killing (36). The kinetics and extent of fractional killing depends on the drug used and its dosage as well as the genetic background of cancer cells (37). On long-term exposure of cells to drugs, a residual population of slow cycling cells, termed as drug-tolerant persisters (DTPs), emerges. These DTPs can subsequently repopulate to the prior heterogeneous population with varying drug sensitivities upon drug removal (38), indicating reversible phenotypic transitions. Investigated in bacterial populations in 1940s (39), DTPs were first reported in cancer in non-small cell lung cancer (NSCLC) cells PC9 which have an oncogenic EGFR exon 19 deletion. Upon treatment of PC9 cells with a lethal dose of EGFR inhibitor erlotinib for 9 days, a small fraction (~0.3%) of the original population survived as slow-cycling DTPs, reflecting existing phenotypic heterogeneity in a population (38). Persistence was observed in other cancer types and in response to other drugs; and single-cell clones established from PC9 cells contained DTPs at a similar frequency as that of parental population, suggesting that DTPs can emerge *de novo*. Importantly, DTPs can

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behave as a reservoir through which heterogeneous (genetic) drug-resistance mechanisms could evolve by allowing cells the opportunity to acquire multiple genetic mutations and/or epigenetic changes (40). Thus, DTPs are canonical examples of phenotypic heterogeneity facilitating a long-term survival. However, many research questions remain mostly unanswered currently: a) what cell-intrinsic and population-level factors control the percentage of DTPs in an isogenic cancer cell population, b) what are some salient molecular attributes of DTPs, c) do DTPs comprise heterogenous subsets, and d) how to effectively target DTPs in the clinic.

Besides DTPs, drug-induced phenotypic plasticity is another trajectory by which cancer cell populations adapt in response to therapeutic stress, as observed by a dynamic transcriptome, metabolome, and epigenome of the surviving cells, independent of additional genomic changes. This evolution follows Lamarckian induction, where the drug itself causes a cell-state change toward a more drug-tolerant state through rewiring of signaling and/or transcriptional networks (41). Importantly, Lamarckian induction is different than Darwinian selection path that is focused on the selection of pre-existing resistant and/or tolerant cells. For instance, treatment of $BRAF^{V600}$ mutant melanoma cells with BRAF inhibitor (vemurafenib) induced a transition of melanocytic (MRAT-1^{pos} $NGFR^{neg}$) cells towards neural crest-like (MRAT-1^{neg} $NGFR^{pos}$) and mesenchymal-like (MRAT-1^{neg} $NGFR^{neg}$) phenotypes in a sequential time-dependent manner. This switch helped some cells to escape the cytotoxic response of the drug, as neural crest-like and mesenchymal-like cell states have relatively higher intrinsic IC50 values for vemurafenib. Thus, BRAF inhibitors can influence cell-state inter-conversion rates among the phenotypes, enriching for a drug-tolerant cell population (41–43). Similarly, treatment of HCC1143 breast cancer cells with either MEK inhibitors or PI3K/mTOR inhibitors enabled cell-state switching to a cell state with reduced proliferation activity and distinct differentiation markers. Despite persisting for weeks under high therapy doses, no specific genomic selection was evident in these residual cells (44). This enrichment of slow-cycling state was shown to be not dependent on selective outgrowth or death of specific subpopulations, by measuring the proliferative tendencies of subpopulations over time. Moreover, the cells that emerged from treatment with PI3K/mTOR and MEK pathways inhibitors were reversible – they regained their sensitivity to the drug, and reconstituted the parental population heterogeneity within 17 days (44), further supporting a mutation-independent evolution. However, the rate of recurrence of slow-cycling cells on next round of drug exposure was not determined, thus, how conserved these dynamics are over multiple treatment cycles remains to the investigated. In another example of Lamarckian induction observed in acute myeloid leukemia (HL60) cells upon treatment with vincristine (10), the frequency of cells exhibiting a drug-tolerant (MDR1^{high}) state increased in a dose-dependent manner – from < 2 % in parental to > 25% in treated population. Within a week after drug removal, the MDR1^{high} cell frequency returned to parental distribution, but the global transcriptome remained relatively unaltered even after 17 days of withdrawal. Together, these observations indicate a drug-induced adaptive and slowly reversible cellular reprogramming.

Single-cell barcoding technologies have further helped delineate the modes of Darwinian selection vs. Lamarckian induction (45,46). In a recent study, barcoded colorectal cancer (CRC) POP66 and CSC28 cells were used to form patient-derived xenograft (PDX) mice models (46). After treatment with irinotecan (CPT-11) once the tumor reached a critical volume, some tumor cells entered a DTP state with reduced proliferation rates (slow cycling). However, the emergence of DTPs did not mark any significant differences in barcode heterogeneity between naïve and drug-treated samples. Instead, the most abundant lineages were random in each drug response study. Similarly, genomic heterogeneity remained conserved during treatment, suggesting a Lamarckian induction in the population instead of selection based on subpopulations/clones fitness differences. The DTPs transcriptome were similar to an embryonic diapause-like state, characterized by slow-cycling and an autophagic response. Thus, an *in vitro* combination treatment of CPT-11 and autophagy inhibitor (ULK1 inhibitor SBI-0206965) showed maximal cellular growth suppression with negligible recovery after drug treatment (46). Other combinatorial treatments that can counteract drug-induced plasticity have been proposed as well by leveraging information about the stability of different phenotypes, their interconversion rates and consequently their population share (47,48). Thus, mapping the longitudinal dynamics of drug-induced plasticity at individual and population levels can help pinpoint actionable therapeutic vulnerabilities.

However, addition of drug can also sometimes drive a transiently resistant transcriptomic state into a stably resistant one through epigenetic reprogramming (9). Therefore, it is important to note that transient and stable modes of drug resistance – and thus, non-genetic and genetic mechanisms – are not mutually exclusive, rather

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semi-dependent phenomenon operating at different timescales (49). Consequently, evading drug response can be a multi-step process mediated through one or more transiently stable drug-tolerant states that can associate with chromatin alterations and can also harbour additional genomic changes under extended periods of therapy-induced stress (38,40,50). What interconnected factors control the extent of reversibility in terms of drug-induced changes remains to be further investigated? The drug-induced changes in population distribution can be envisaged as a new dynamic equilibrium (51); for instance, the population can enter an "idling" state such that the number of cell division and cell death events are equal, thus maintaining a population size (**Fig 3C-E**). Upon withdrawal of drug, population heterogeneity can increase again, as drug-sensitive states are repopulated, reminiscent of observations seen in DTPs (**Fig 3F**).

Overall, an interplay among the emergent dynamics of intra-cellular regulatory networks (52), therapy-induced plasticity (41), and group behavior among the diverse phenotypes in a cell population (35), can shape population heterogeneity patterns.

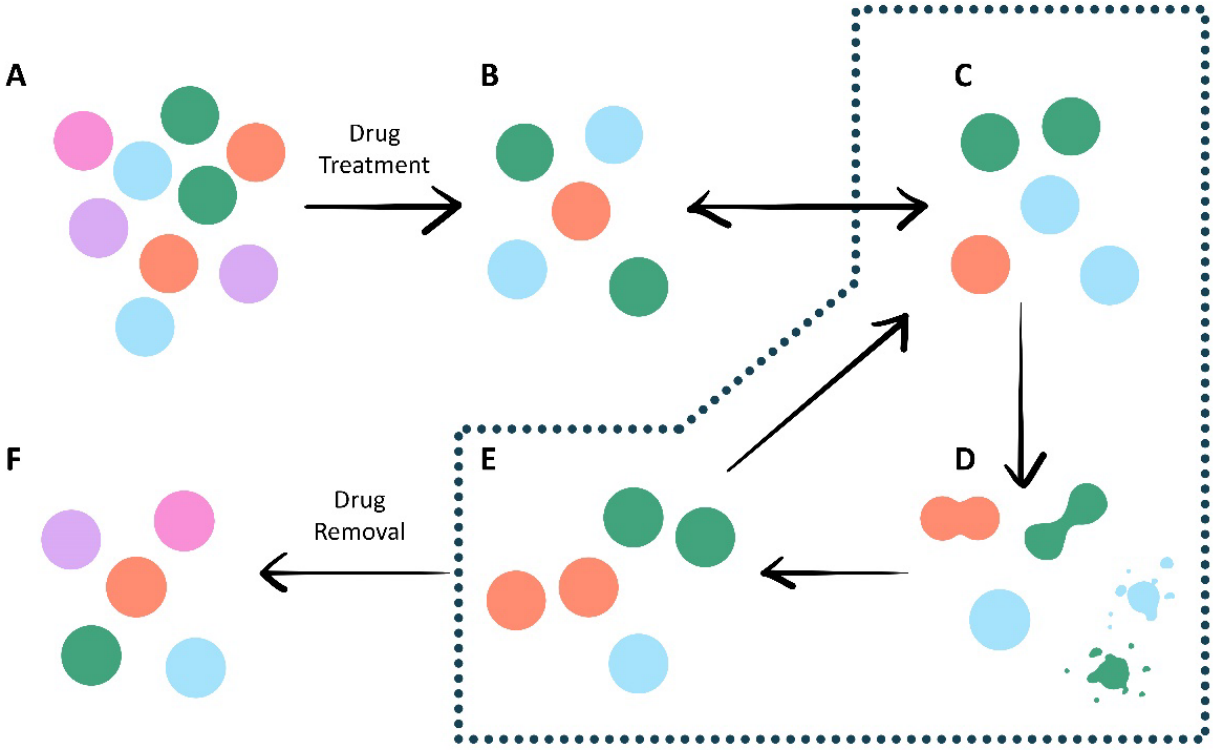

***Fig. 3. Drug induced cell-state transition**. **A)** Heterogeneous cell population with cells spontaneously switching between drug sensitive (purple, and pink) and resistant (green, orange and blue) states. **B)** When the population encounters drug treatment, pre-existing resistant cells or those that can switch to a resistant state survive. **C-E)** the survivor cells enter an idling population state where the population size remains constant with number of cell divisions equalling cell deaths. **F)** Upon drug release, the population's cell-cell variability enhances as some cells may re-enter drug sensitive states.*

Mathematical models as a tool to understand cell-state transitions in cancer

Several above-mentioned studies have incorporated a mathematical modelling approach to better understand the dynamics of cell-state transitions at individual cell as well as at population levels. These models have been instrumental to quantify cell-state transition rates from one subpopulation to another (17,20), explain population dynamics behavior in the absence and presence of drug (10,42,51), and to predict sequential therapies that can be helpful in overcoming the drug-induced plasticity in a cell population (23).

At a cell population level, Markov Chain (MC) model is an extensively employed approach to study spontaneous/drug induced cell-state transitions (17,20,41). Using the population fraction of cellular states over

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time, MC model can determines the transition probabilities between each pair of states (**Fig 4A**). These transition probabilities are considered to be independent of time, and therefore, depend only on current state distribution of the population, and not on its previous history. These inferred transition probabilities can then be used to predict long-term behavior of a cell population with a known initial phenotypic distribution. These predictions can be directly tested experimentally. For instance, MC model inferred the rates of self-renewal and interconversion among the luminal, basal and stem-like subpopulations in SUM159. A striking prediction of this model was that both the luminal and basal cell-states had a non-zero probability of switching to stem-like states; in other words, non-CSCs could stochastically switch to CSCs *de novo*. This prediction was validated *in vivo* where both the luminal and basal sub-populations could regenerate functional stem-like cells (17). Similarly, the transition rates inferred for MC model applied to repopulation dynamics data from four different subsets of CSC ($CD24^{high}$ $ALDH^{high}$, $CD24^{high}$ $ALDH1^{low}$, $CD24^{low}ALDH^{high}$ and $CD24^{low}$ $ALDH^{low}$) indicated that not all CSC subpopulations could regenerate one another. While the $CD24^{high}$ sub-populations could not generate $CD24^{low}$ cells, $ALDH^{high}$ and $ALDH^{low}$ ones could interconvert, highlighting a possible hierarchy within the diverse CSCs. Drug treatment can alter the transition rates between $ALDH^{high}$ and $ALDH^{low}$ to drive enrichment of $ALDH^{high}$ cells (20). Further, MC models can be modified to include additional factors such as cell growth (21) or differential viabilities of cell populations to specific drugs. For instance, such a modified MC model revealed that the ~5-fold increase in stem-like and basal cells upon paclitaxel treatment was not due to selection of basal cells, but increased viability of stem-like cells that could then switch to basal cell state (17). Such dynamic insights can be valuable in deciding therapeutic strategies to reduce phenotypic plasticity and/or heterogeneity in a cancer cell population.

Ordinary Differential Equation (ODE) based models represent another general class of approaches to track population dynamics (10,53). Similar to MC models, they often capture the mean behaviour of a population. For instance, an ODE-based model for population dynamics of sensitive and resistant cells in MDA-MB-231 cells was calibrated using clonally resolved single-cell RNA-seq data and bulk longitudinal population growth data during treatment with doxorubicin (53). This calibration helped the model to predict population dynamics at several drug concentrations (**Fig 4B**). Also, ODE-based models can be crucial in unravelling the intracellular dynamics of signaling molecules; for instance, different ODE-based models for EMT regulatory networks predicted that EMT was not a binary process, instead cells can attain one or more hybrid E/M states (54–56). These hybrid E/M states have been since identified *in vitro* and *in vivo* across multiple cancer types (57–59). Similar models also predicted the association of hybrid E/M phenotypes with enhanced tumor-initiation potential and immune-evasive traits (60,61), predictions that have been experimentally validated (30,62). Further, mechanism-based ODE models for EMT networks have suggested the existence of hysteretic dynamics, i.e. cells take a different path for undergoing EMT vs. MET (**Fig 4C**) (63). Such dynamics have been experimentally observed at transcriptomic and proteomic levels (64,65). These examples showcase how mathematical models can contribute toward identifying relevant cell-state capable of accelerating disease aggressiveness, and demonstrate the salient dynamical hallmarks of cellular transitions.

Besides MC and ODE-based models, stochastic simulations at intracellular and/or population level have been instrumental in unravelling regulatory mechanisms driving cancer cell behavior. For instance, stochastic multi-scale models incorporating asymmetric partitioning of molecules during cell division (66,67) could reproduce experimentally observed temporal dynamics of switching among epithelial, mesenchymal and hybrid E/M phenotypes in prostate cancer PKV cells (28)and breast cancer PMC42-LA cells (27). Similarly, a stochastic model for the emergence of DTPs was used to understand the clone size distribution data obtained by longitudinal barcoding (tracking of individual cancer cells) from both the untreated (control) and chemotherapy-treated groups (46). This model predicted that each cancer cell was equipotent in reversibly switch to being a DTP, thus endorsing observations from population dynamical models demonstrating that non-genetic factors such as stochastic gene expression and associated phenotypic switching can drive long-term resistance to many drugs even in absence of any *bona fide* resistance modes (68,69). Last but certainly not the least, stochastic models can also help identify the trajectories cells take as they transition among different phenotypes, and suggest effective “state-gating” strategies to restrict exploration in phenotypic plasticity landscape (47,48). Overall, stochastic models have been instrumental in deciphering the underlying fundamental dynamical principles of cancer cell population behavior, and can help leverage this better understanding to design smarter therapies.

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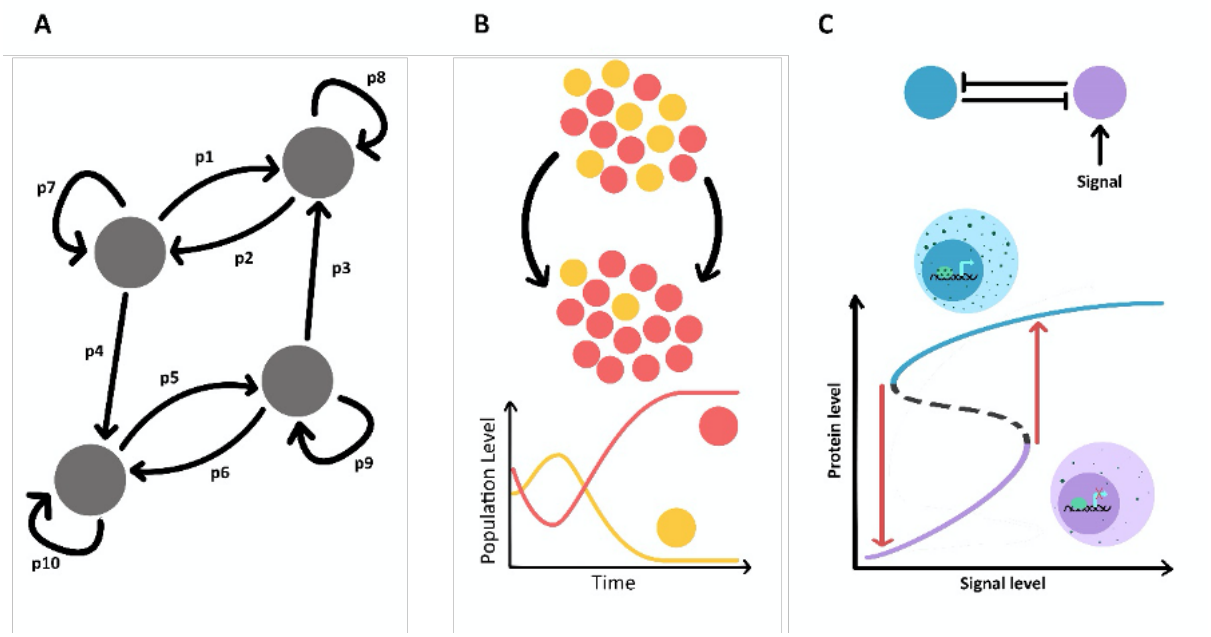

Fig 4. Depiction of modelling strategies to capture spontaneous and drug-induced state switching*. **A)** Markov chain state transition models – accounts for transition between states by quantifying transition probabilities at each simulation step. Possible transitions between states are depicted by an arrow from one cell state to another with p1, p2...p10 as transition probabilities. **B)** ODE-based population models – defines a phenotype abundance as dependent variable of time and the rate equations accounts for increase or decrease in the phenotype's abundance due to proliferation, death, and state switching. **C)** Mechanism-based models – bifurcation diagram resulting from interaction between regulatory players. The two stable expression states, low (purple curve) and high (blue curve), of a regulatory player (along y-axis) are based on levels of signal (along x-axis). The red arrows depict state switching when signal crosses a threshold level and highlight hysteretic behaviour.*

Summary

- Phenotypic plasticity and heterogeneity in tumors can exist along multiple interconnected axes: CSCs/non-CSCs, epithelial-mesenchymal plasticity, drug-tolerant/drug-sensitive cell-states.

- Intratumor heterogeneity can emerge from non-mutational mechanisms too – stochastic gene expression, chromatin reprogramming, asymmetric cell division and the presence of multiple "attractors" – that can drive spontaneous and/or externally induced cell-state transitions.

- The rates of transition among cell-states, and consequently reversibility of a phenotypic switch, depends on many factors: dose and duration of inducing signal, initial population distribution, and relative stability of different "attractors" corresponding to distinct cellular phenotypes.

- Both Darwinian selection (selection of the pre-existing 'fitter' cells) and Lamarckian induction (drug-induced changes in cellular phenotypes) can impact the evolution of a heterogeneous cell population under stress.

- Mathematical models can infer cell-state transition rates and predict long-term population-level behavior under varied conditions, such as therapeutic stress.

Competing Interests

The authors declare no conflict of interest.

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Author contributions

All authors contributed in writing and editing the manuscript.

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