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Review

Systems biology

Single-cell systems analysis: decision geometry in outliers

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Abstract

Motivation: Anti-cancer therapeutics of the highest calibre currently focus on combinatorial targeting of specific oncoproteins and tumour suppressors. Clinical relapse depends upon intratumoral heterogeneity which serves as substrate variation during evolution of resistance to therapeutic regimens.

Results: The present review advocates single-cell systems biology as the optimal level of analysis for remediation of clinical relapse. Graph theory approaches to understanding decision-making in single cells may be abstracted one level further, to the geometry of decision-making in outlier cells, in order to define evolution-resistant cancer biomarkers. Systems biologists currently working with omics data are invited to consider phase portrait analysis as a mediator between graph theory and deep learning approaches. Perhaps counter-intuitively, the tangible clinical needs of cancer patients may depend upon the adoption of higher level mathematical abstractions of cancer biology.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Cancer outliers

The USA overall cancer mortality rate decreased 25% over an equivalent number of years (Siegel *et al.*, 2017). Owing to the outstanding known-unknowns, recalibration of public expectation towards containment of cancer, has been suggested as a cultural norm (Tiwari *et al.*, 2012). An alternative perspective reasons that utilization of a different conceptual approach will result in a different rate of mortality reduction.

Current conceptual paradigms underpinning clinical practice centre on combination regimens of targeted molecular therapeutics (Aebersold *et al.*, 2009; Gotwals *et al.*, 2017). Drug combinations are superior to monotherapies (Jia *et al.*, 2009) in reducing host toxicity, since dosages of drug combinations are typically lower when compared with dosages of single agents (O'Neil *et al.*, 2016). However, drug combination effects can be adverse and even lead to shorter progression-free survival of cancer patients (Hecht *et al.*, 2009; Preuer *et al.*, 2018; Tol *et al.*, 2009). Preuer *et al.* (2018) have applied deep learning techniques to pharmaco-synergy prediction in order to improve the efficacy of anticancer treatments. Relapse rates remain high (Milojkovic and Apperley, 2009) due to clonal evolution of resistance across targeted therapies (Aebersold *et al.*, 2009; Dagogo-Jack and Shaw, 2018; Kalmanti *et al.*, 2015; Tiwari *et al.*, 2012). Variation, upon which natural selection acts to drive the evolution of chemotherapeutic resistance, manifests as heterogeneity between individual cells (Dagogo-Jack and Shaw, 2018). However, whole-cell population studies are blind to clinically relevant intercellular heterogeneity (Wang and Bodovitz, 2010). In contrast to whole-cell population studies, single-cell biology provides the requisite lens to untangle the evolution of chemotherapeutic resistance. Indeed, the single cell represents the highest resolution biological unit (Lubeck, 2016) that retains the capacity to manifest all six hallmarks of cancer (Hanahan and Weinberg, 2011).

In tandem, systems biology integrates the phenomenological approach of physiology with the mechanistic (Fig. 1) approach of molecular biology (Bhalla and Iyengar, 1999, Wist *et al.*, 2009). Systems analysis transcends the reductionism inherent in most biological research approaches (Bhalla and Iyengar, 1999; Ehsani, 2018) rendering explicable the emergence of properties that effect cell fate decision-making (Aebersold *et al.*, 2009; Weng, 1999). As such, systems biologists have the visionary insight to recognize

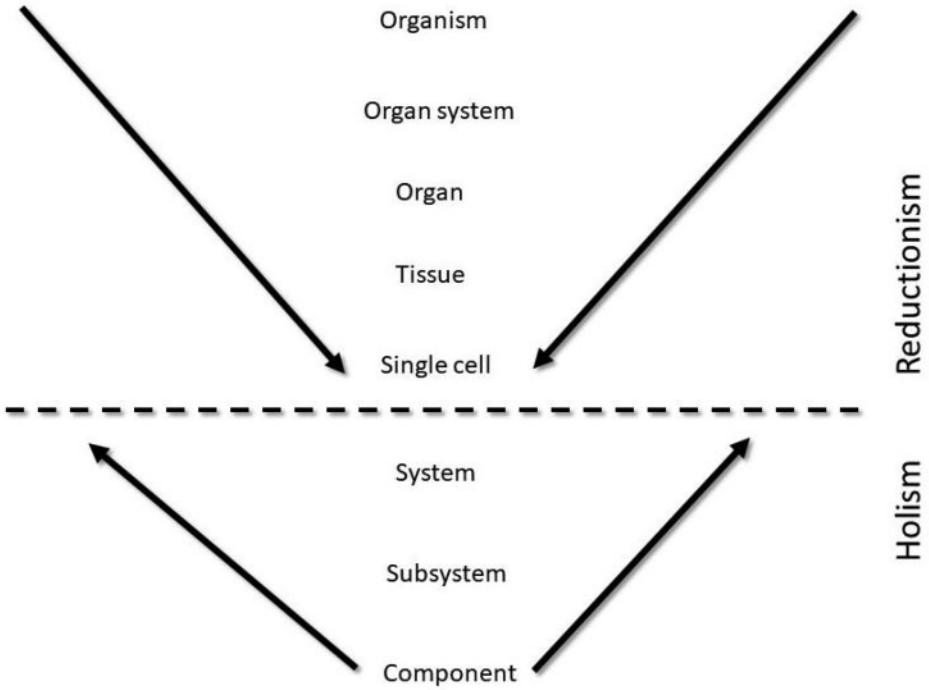

Fig. 1. Single-cell systems biology liaises reductionism, inherent in study of the highest resolution biological unit capable of manifesting all six hallmarks of cancer, with the holism characteristic of systems level analysis. Upper panel: descending the hierarchy of biological organization is a mainstream strategy for reducing complexity, although the co-efficient of variation is self-similar at all levels of biological hierarchy (Sapolsky and Balt, 1996), suggesting that the decision code may ultimately be fractal in nature. Lower panel: ascending the hierarchy of biological analysis from study of single components to whole systems

systems as the optimal level of biological abstraction (Patange *et al.*, 2018). Unification of two inquiry fields into single-cell systems analysis grants the possibility to comprehend the rarest cells of greatest malign that reverse clinical success.

For the purposes of the present review, outlier cells are defined as single cells that are: (i) neither entirely physiologically normal nor expressing macroscopic hallmarks of cancer; or, (ii) transformed cells that are significantly different to the majority cell populations within the tumour. Outlier cells are understood to be transitional or intermediate cell states, as expounded by MacLean (2018), that exist on the edge of transformation or resistance and which manifest aberrations at the level of information processing. Herein, the reader will receive a new way of defining outlier cells, and will understand the rationale for utilizing 6D-phase portrait analysis in preference to expression analysis.

The clinical urgency to correctly identify outlier cells is corroborated by a number of studies that have achieved this feat. Four rare tumor cells, absent in the major tumour subpopulations, exhibited 50-fold amplification of the KRAS locus (Fearon and Vogelstein, 1990; Navin, 2014) and most malignant populations in a tumour are likely to be the rarest (Navin, 2014). A tumour of mass one gram contains approximately 1×10^9 cells (Del Monte, 2009); every conceivable subclonal mutation could exist in at least one cell in the tumour (Schmitt *et al.*, 2016). Further, single-cell sequencing evidence implies most human tumours originate from single cells in the normal tissue (Navin, 2014). Furthermore, rare disease-specific subpopulations of microglia have been identified exclusively via single-cell systems analysis (Keren-Shaul *et al.*, 2017). More than a decade of population-based assays, including cell sorting using specific cell-surface markers and bulk RNA sequencing, failed to flag these cells (Keren-Shaul *et al.*, 2017).

Single cells display considerable intercellular variation in expression levels of individual biomolecules (Prakadan *et al.*, 2017), hereby referred to as vertices, with respect to their functional role in regulatory networks. The central difficulty with identifying outlier cells is the requirement to distinguish cells on the basis of behavioural patterns. Cancer cells archetypally manifest six distinct hallmark behaviours: (i) sustaining proliferative signalling, (ii) evading growth suppressors, (iii) resisting cell death, (iv) enabling replicative immortality, (v) inducing angiogeneis; and, (vi) activating invasion and metastasis (Hanahan and Weinberg, 2011). Hallmarks of cancer are the outcome of cellular decisions to transition between binary states: (i) constitutive versus non-constitutive growth factor signalling, (ii) passage versus non-passage of G1 checkpoint, (iii) cell survival versus cell death, (iv) immortality versus senescence, (v) erection versus absence of vasculature; and, (vi) extravasation versus immobility. Two novel approaches to capturing single cells or subpopulations on the basis of macroscopic behaviours have been advanced recently. One approach captures single cells from a heterogeneous cell culture on the basis of motility behaviour (Desjardins-Lecavalier *et al.*, 2020). The second approach captures chemo-resistant subclones from heterogeneous cancers via CRISPR barcoding of >1 million cells (Zhang *et al.*, 2019). The latter work is of great interest and would solve the core problem posed herein. However, the work is preliminary and as yet only available as an abstract. Moreover, CRISPR technology is invasive and not currently suitable for clinical use. As such, the current review aims to make available to the reader a greater selection of arguments within the marketplace of ideas.

Harnessing correlations between binary states and whole-cell population based averages of vertex expression levels, vertices morphed into proxies of behavioural patterns. Notwithstanding, any given single cell that appears to be an outlier in terms of static

expression levels of vertices may not be functionally an outlier; distinct connectivity patterns can elicit the same functionality (Buchbinder *et al.*, 2018; Miller, 2016). Problematically, conventional approaches are predicated on the axiom that distinct expression patterns invariably indicate distinct functionalities. As noted by Giladi and Amit (2017), assumptions about cellular states may be shed, in order to rebuild representations of cellular networks (Giladi and Amit, 2017).

2 Vertices to subgraphs

2.1 Decision fragility on subgraph negation

Graph theory offers the means to supplant assumptions relating to cellular states (Giladi and Amit, 2017) and to re-imagine the nature of behavioural patterns in outliers. Single cells, as self-contained units of decentralized self-organising behaviour, prompt questions regarding the level at which decisions are made within the hierarchy of network topology.

Subgraphs are a leading candidate as the optimal unit of analysis in network topology (Berger and Iyengar, 2009; Ma'ayan *et al.*, 2005); scale-free networks are anti-fragile to removal of individual vertices while being fragile to disruption of hubs (Callaway *et al.*, 2000). Perturbation analysis is typically utilized to pinpoint biological entities for which removal renders the functionality of a system fragile (Keenan *et al.*, 2018). Fragility of biological networks to removal of hubs, contrasted with robustness to removal of individual vertices, implies functionally significant decision-making logic is encrypted at the level of subgraphs. Subgraphs significantly associated with survival have been found, containing vertices previously reported to be important for cancer prognosis, in which no single vertex is associated with survival when considered in isolation (Hansen and Vandin, 2016). Synergy may explain the competitive edge of subgraph analysis over the orthodox consideration of individual vertices or whole graphs (Erten *et al.*, 2012; Hansen and Vandin, 2016). Indeed, subgraph analyses are capable of informing cancer diagnosis, clinical staging, prognosis and biomarker identification as summarized in Supplementary Table S1. Despite the empirical importance of subgraphs in determining cell fate decisions, subgraph analyses currently have predictive accuracy in the range of 65-91% (ST2). As an exemplar, arguably the most comprehensive and well-annotated whole cell computational model in existence (Karr *et al.*, 2012), modelled on 1900 parameters derived from 900 research publications, has a predictive accuracy gap of 33%. Almost invariably, the foregone conclusion is that any gap in predictive efficacy must be causally related to deficiencies in the scope and depth of parameterization within the model. Alternatively, the predictability gap may reflect requirement for further mathematical idealization.

Subgraph identification is a young field of biological analysis; while there are leading exemplars in the literature (Bartlett *et al.*, 2017; Choobdar *et al.*, 2019) discovery potential remains high. In addition, currently available literature on subgraph analysis invariably converge on regulatory enzyme expression data (Supplementary Information S11) rather than regulatory enzyme activity data, meaning that any subgraph activation is inferred rather than explicitly proven. As such, the niche area of subgraph activity assay has high discovery potential. It is proposed that the nature of behavioural patterns in outliers be investigated initially by means of analysis of all possible combinations of subgraph activation ratios, derived from absolute values of subgraph firing in single cells, within discrete time intervals. In particular, creation of an index or metric derived from subgraph activity data, would compress into a useable form information relating to the relative ratio of 6 hallmark behavioural patterns. Kim *et al.* (2017) provide proof-of-concept for this type of metric, although Kim *et al.* (2017) focus specifically on three hallmarks (proliferation, EMT and stemness) whereas the current ambition seeks a fully comprehensive metric.

Definition of subgraphs may be apriori or aposteriori (Supplementary Information S1; Kim *et al.*, 2017); the former utilizing the substantial body of knowledge already available in the literature and the latter leveraging unsupervised deep-learning (Vandin *et al.*, 2012). Parallel pursuit of each approach with critical comparison of predictive efficacy may elicit maximal value.

2.2 Subgraph activation ratios

A comprehensive overview of single-cell techniques is beyond the scope of the current review and interested readers are referred to Stuart and Satija (2019). In Supplementary Table S3 and Supplementary Information, we briefly consider current prospects in relation to laboratory technologies, in order to allow readers to decide on a preferred means of investigating subgraph activation in their own laboratories (Supplementary Information S2). The next section discusses fractal decision-making and outlines how subgraph activation may be translated into phase portraits in order to assay the holistic state of a single-cell system.

3 Subgraphs to phase portraits

3.1 Dynamical systems

A leading known-unknown in the field of single-cell systems analysis is the precise degree to which decision-making architecture is sensitive to initial conditions. Delineation of sensitivity to initial conditions has critical implications for the extent to which cell fate decisions are predictable and, therefore, the extent to which scientific forecasting of cell fate decisions is feasible. The conventional argument, taken to its logical conclusion, posits that the absolute copy number of each of the $>2.0\times10^9$ (Milo, 2013) vertices in an individual cell effects cellular behaviour in a reasonably deterministic manner.

In essence, predictability depends upon the extent to which the system is ordered or disordered. Fully ordered systems are deterministic whereas fully disordered systems are entropic. Deterministic systems are predictable whereas entropic systems are unpredictable. Cancer cells are entropic (West *et al.*, 2012) relative to untransformed cells; Berretta and Moscato (2010) advocate normalised Shannon Entropy measures as a unifying hallmark of cancer. Information Theory approach to identifying outlier cells is intriguing for two reasons. Firstly, the entropy hallmark is upstream of all six Weinbergian hallmarks of cancer, meaning that the entropy hallmark will capture all of the transformed cells exhibiting macroscopically disordered phenotypes. Secondly, the entropy hallmark flags transitional outlier cells exhibiting disordered patterns of behaviour at the level of information processing. Conventional screening on the basis of the six macroscopic hallmarks of cancer would overlook transitional outlier cells; one source of chemotherapeutic resistance.

3.2 Fractal decision-making

Since the lethality of cancer may be explained at the thermodynamic level as an unfurling of systemic disorder, normal physiological cell systems may intuitively be expected to be highly ordered. In actuality, normal physiological cells are not entirely deterministic; non-transformed cells are non-linear dynamical systems observed to

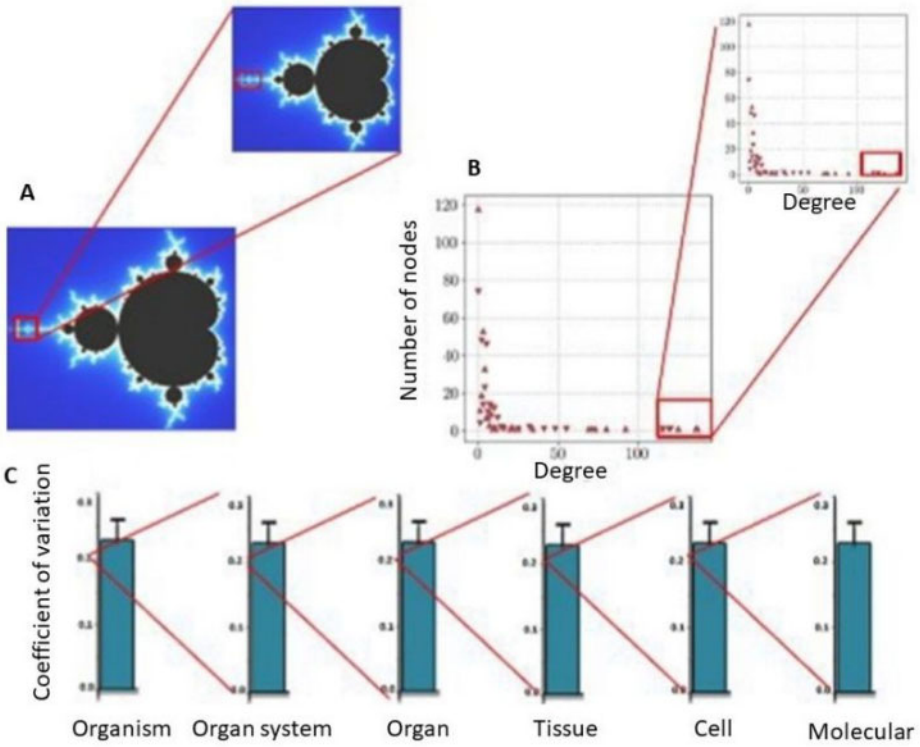

Fig. 2. Fractal decision-making. (**A**) Fractals are observed in the Mandelbrot set; note the appearance of self-similar features (red panel) at multiple scales. Adapted from Liverpool Mathematical Society. (**B**) Zooming in on a subset of highly connected vertices within a graph (red panel), the degree distribution of the selected subset is equivalent to the original, and therefore independent of scale (Mitchell, 2018). (**C**) Interestingly, the co-efficient of variation is self-similar at all levels of biological organization (Sapolsky, 2016), which suggests the decision-making code may be inherently fractal. Note that the bar chart data are illustrative rather than data as exactly reported; original data are available in Sapolsky and Balt. Adapted from Sapolsky and Balt (1996)

transition between states of equilibrium, periodicity (Mullassery *et al.*, 2008), quasi-periodicity and deterministic chaos (Miller, 2016; Sharma, 2009). Plasticity to transition between highly ordered and highly flexible states may prove to be an evolutionarily stable strategy, conferred upon cells by natural selection, in order to synchronize decisions simultaneously across multiple levels of biological organization and multiple timescales. Decisions made at the organismal, organ system, organ and tissue level impact the fate of single cells; likewise, decisions made at the single-cell level impact the fate of tissues, organs, organ systems and organisms. Decisions made across chronic time scales impact acute cell fate; likewise, acute decisions impact longitudinal cell fate. As an exemplar, spiral arrhythmia in the heart appears random at the cellular level, but is ordered at the level of the whole organ (Kharchenko *et al.*, 2014). Interestingly, biological systems manifest self-similarity across multiple scales of organization; a fractal property (Fig. 2).

For example, biological networks typically feature a minority of very highly connected vertices while the vast majority of vertices have low connectivity; the degree distribution follows the power law (Ma'ayan *et al.*, 2005; Sapolsky and Balt, 1996; Sharma, 2009). Graphing of the degree distribution yields a long-tailed distribution. Of particular interest, honing in on the minority of highly connected vertices and investigating their degree distribution as a separate sample of vertices, results in a self-similar long-tailed distribution. Degree distributions in biological networks are independent of scale and, therefore, scale-free; a fractal property. Moreover, Sapolsky and Balt (1996) conducted a meta-analysis to test the ubiquitous assumption that increasingly reductionist approaches to biological study result in decreasingly noisy data. Investigating the particular question of testosterone-driven impact on behaviour, Sapolsky and Balt (1996) approached the biological question at increasingly reductionist levels, beginning with anthropological studies through to X-ray crystallography studies of individual testosterone receptors. Meta-analysis suggests that the co-efficient of variation remains constant across multiple levels of biological organization, meaning that the degree of variability is independent of the scale of observation; a fractal property.

Single cells, having a decision architecture characterized by fractal properties (Fig. 2), invite consideration of the geometry of decision-making in outliers. Consider again the prospect of observing and plotting six hallmark parameters over time, an argument advanced in the preceding segment of this review. Rather than plotting each parameter separately with time as the independent variable, the geometry of decision-making in outlier cells may be revealed with clarity by plotting all six parameters on multidimensional axes (Fig. 3), in which each axis corresponds to one of the coordinates required to specify the state of the system. All of the coordinates being thus represented, a point in the multidimensional phase space would correspond to one state of the system. Geometric analysis would therefore serve the aforementioned (Giladi and Amit, 2017) meta-purpose of reimagining the states of cellular systems.

3.3 Patterns of behaviour

Tracking the path traced over time by the evolving state of the system yields a trajectory. Trajectories are real patterns of behaviour and therefore fulfil the primary criterion as a means of identifying transitional outlier cells that confer chemotherapeutic resistance. Further, plotting multiple single-cell trajectories, each corresponding

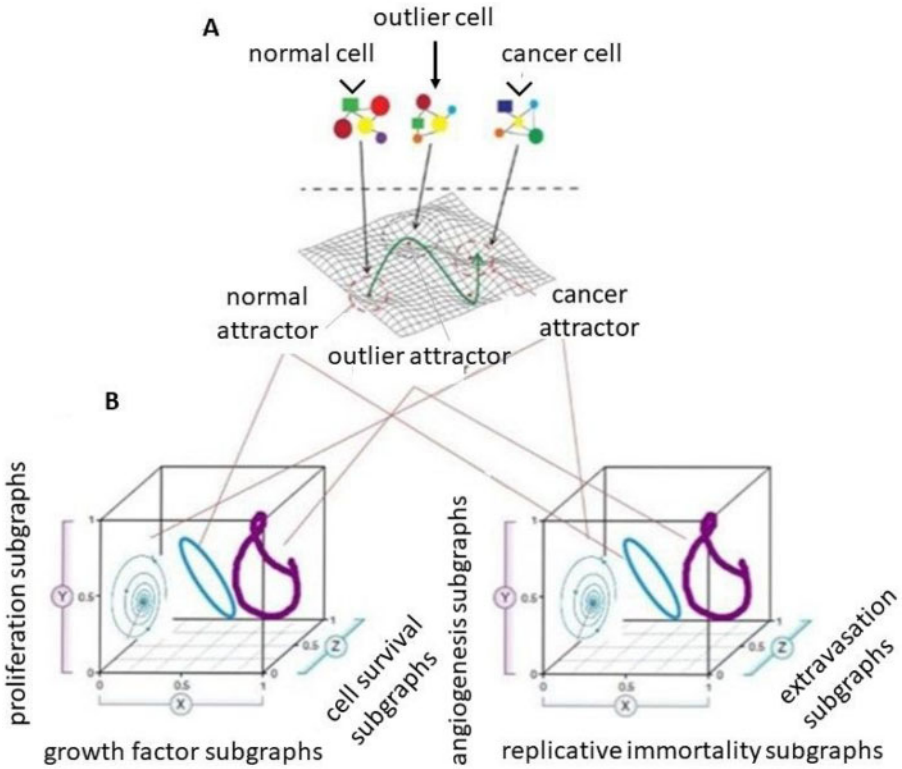

Fig. 3. Subgraphs to phase portrait analysis. (**A**) Analysis of subgraph activation ratios, along six dimensions of behaviour, can be plotted on multidimensional axes (**B**) with the aid of dimensionality reduction approaches. Imagining a 2D plane of subgraph activation, viewing of the time series straight down the time axis yields a trajectory (green). In this example, the trajectory intersects one or more stable attractor states. (A) Adapted from Fard and Ragan (2017). (B) Adapted from Koseska and Bastiaens (2017)

to one set of initial conditions internal to a single cell, in the same phase plane will produce a phase portrait. Phase portraits capture the systems physiognomy of single cells and are amenable to efficient identification of rogue trajectories (Fig. 3). Moreover, phase portrait analysis reveals the existence of any attractors present in the system. Attractors are stable, low entropy states of a dynamical system, towards which a particular set of points in phase space evolve over time (Bornholdt, 2008; Hirsh *et al.*, 2012; Sharma, 2009; Wuensche, 1994).

Normal cells and cancer cells alike harbour attractor landscapes (Fig. 3) and the process of cellular transformation can be conceptualized as the process of normal cells sliding irreversibly into stable cancer attractor states (Kim *et al.*, 2017; Koseska and Bastiaens, 2017; Maetschke and Ragan, 2014). Kim *et al.* (2017) developed a sophisticated scoring system for the attractor landscape, derived from a combinatorial state of 8 marker vertices, in order to distinguish normal attractors from cancer attractors. Three synergistic vertex pairs are identified as a result of the node perturbation analysis which may inform combinatorial pharmacological targeting. However, the utility of combinatorial targeting is threatened by the development of multidrug resistance. Moreover, an equivalent identification of synergistic vertex pairs may be obtained by investigation on the basis of macroscopic hallmarks of cancer, without recourse to attractor analysis. In lieu of this particular application of attractor landscape analysis, it is proposed that the strength of Kim *et al.*'s (2017) scoring system be first enhanced by expanding the hallmark selection to include all six Weinbergian hallmarks. Secondly, the current review proposes that in order to maximize the utility of attractor landscape analysis, the approach be applied to the endeavour of identifying transitional outlier cells implicated in the evolution of chemotherapeutic resistance. Attractor landscape analysis, in the context of outlier cells, is necessary and impossible to supersede with study of macroscopic hallmarks of cancer. Transitional outlier cells do not yet exhibit macroscopic hallmarks of cancer and their identification is wholly contingent upon detection of patterns of behaviour in information processing.

Returning to the topic of information processing, cellular decision-making has been likened to a cognition (Koseska and Bastiaens, 2017) and patterns of habitual responding in cellular decision-making may reflect a deep attractor basin analogous to the deep attractor basin of neural networks (Hirsh *et al.*, 2012). In the next and penultimate section of this review, single cells are appraised as naturally intelligent and autonomous arbiters; the nature of single cells as deep learning information processors has important ramifications for designing evolution-resistant therapies, as delineated in the final analysis.

4 Deep learning: single cells

4.1 Single cells as intelligent entities

Intelligence of single cells has been commended by multiple scientists (Albrecht-Buehler, 1985; Ford, 2009; 2017; Gerrard *et al.*, 2014; Ghosh, 2018; Tero *et al.*, 2010) on the basis of their ability to: (i) solve the Travelling Salesman Problem (Tero *et al.*, 2010), (ii) construct external shells as an extended phenotype (Ford, 2009, 2017), (iii) perform cellular memory (Ford, 2009, 2017), (iv) execute autonomous decision-making (Ford, 2009, 2017; Ghosh, 2018; Tero *et al.*, 2010); and, (v) manifest computational properties in cell

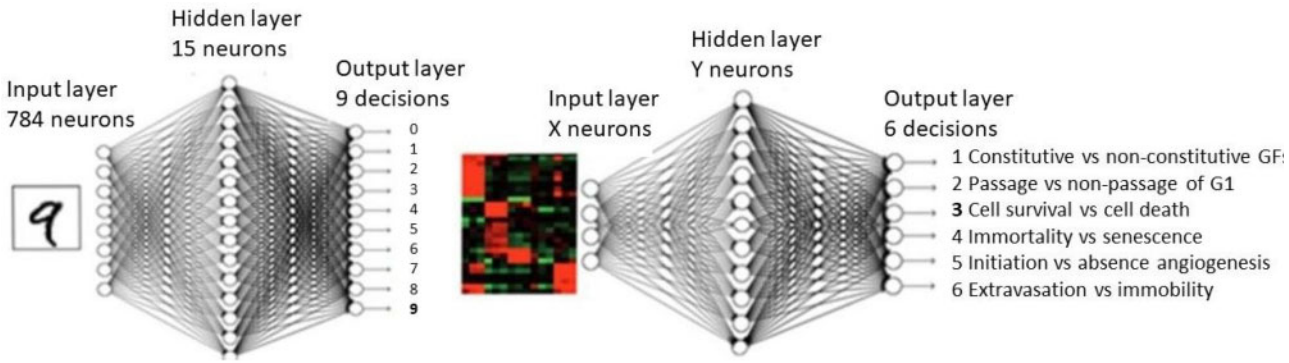

Fig. 4. Critical comparison of the decision architecture in artificially and naturally intelligent systems. Upper panel: artificial neural networks make a 1-in-9 decision on the basis of over 3000 differentially weighted and biased connections between neurons. Lower panel: representing the subgraph activation status of a single cell as a heatmap to be decoded, in a similar manner to the handwritten digit image, may expedite understanding of decision-making in single cells. Adapted from Nielsen (2019)

signalling (Gerrard *et al.*, 2014; Ghosh, 2018; Tero *et al.*, 2010). Re-estimation of single cells as naturally intelligent and autonomous agents may catalyse understanding of the nature of cell fate decision-making (Supplementary Information S3). In many respects, cellular decision architecture is structurally and functionally analogous to neural networks. Neural circuits and cell decision circuits alike are non-linear dynamical systems that can be described by coupled differential equations (Miller, 2016). To take the example of artificial neural networks that recognize handwritten digits, the task of decision-making (selecting correct digit between 0 and 9) is facilitated by multiple hidden layers of neurones flanked by input and output layers of neurons. By way of analogy, biological cells are tasked with decision-making (selecting correct cell fate along 6 Weinbergian axes) that is facilitated by multilayer networks. Interestingly, decision-making architecture in single cells may readily be represented as a series of temporally connected heatmaps (Fig. 4) in which each pixel of the heatmap is informed by non-binary subgraph activation ratios at distinct time points. In this scenario, single cells are modelled as if equivalent to individual convolutional neural networks, each with 6 decision outputs.

Digit recognition neural networks have 96% accuracy while the gold standard within artificial neural networks is 99.79%. Given that the fidelity of decision-making in single cells is estimated to outcompete the fidelity of decision-making in neural networks (Supplementary Information S4), single cells may reasonably be interpreted as having comparable intelligence to multilayer perceptrons.

4.2 Deep learning on phase portraits

Rather than attempting the impossibly labyrinthine task of utilizing precision medicine on every individual outlier cell (Supplementary Information S5), this review advances the case for in vivo targeting of transitional outlier cells on the basis of one universal (Supplementary Information S6) and evolution-proof flag: the 6-dimensional phase portrait. As aforementioned, the 6-dimensional phase portrait, a visualization of global patterns of decision-making behaviour, captures the systems physiognomy of outlier cells. Outlier cells have a distinct phase portrait; decision-making patterns of behaviour are dysregulated relative to both untransformed and chemo-sensitive cells. Moreover, of the conventional targets utilized in cancer that fail, the majority fail due to clonal evolution of a non-transformed biomarker expression profile; cells evade detection via masking. Unlike conventional targets, 6-dimensional phase portraits capture global patterns of decision-making behaviour in single cells, in a manner that is robust to up- or down-regulation of one or a few vertices, and is therefore robust against clinical oversight of cells on the edge of transformation. As a target, 6-dimensional phase portraits are resilient to evolution of the masking phenomenon. If outlier cells were to evolve a non-dysregulated phase portrait mask in response to selective pressures, essentially the global patterns of decision-making behaviour in single outlier cells would revert to normal, and the reserves of chemotherapeutic resistance-bearing outlier cells would diminish (Supplementary Information S7).

4.3 Unsupervised feature extraction

Pragmatically, an adjuvant therapeutic agent designed to identify subpopulations of single outlier cells at high risk of conferring chemotherapeutic resistance, will necessitate dimensionality reduction (Fig. 5) in order to extrapolate backwards and extract signature features from phase portraits. While the overall mission may seem tautological on first inspection—constructing a global portrait of behaviour in order to subsequently deconstruct in pursuit of a minority of defining outlier features—three core arguments justify the construction-deconstruction rationale.

Firstly, transitional outlier cells do not necessarily manifest the macroscopic hallmarks signatory of transformed cells, although information processing through decision-making apparatus is typically aberrant. Therefore, attempting to extrapolate backwards from single cells that display macroscopic hallmarks of cancer will fail. Secondly, single outlier cells by definition are highly heterogeneous—displaying high variability even within the subset of outlier cells—in terms of expression and activity of individual vertices. Therefore, any expedition to extract features without reference to universal phase portrait analysis, would require that a therapeutic agent be targeted against multiple different targets and be capable of detecting cellular profiles on an individual basis, in vivo and in real time. Universal phase portrait analysis overcomes the high-heterogeneity paradox. Thirdly, universal phase portrait analysis illuminates emergent patterns of behaviour, not immediately apparent at more reductionist levels of analysis. Therefore, it is more efficient to extrapolate backwards from a subpopulation of single cells that manifest a universal phase portrait, than it is to extrapolate forward from highly heterogeneous profiles of vertex expression.

Deep learning can assist with both construction and deconstruction phases, although principally in silico learning is anticipated to be of maximal value in the latter phase. As noted by Coudray *et al.*

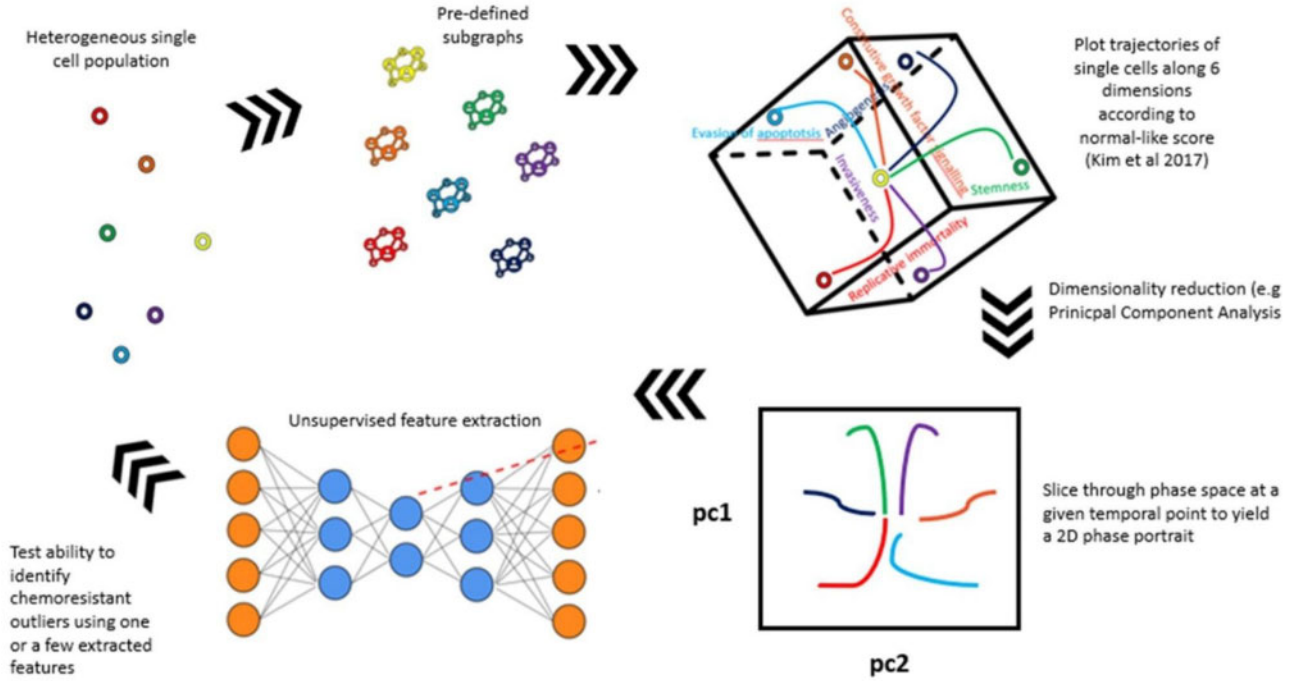

Fig. 5. Schematic overview of the proposed workflow to identify chemo-resistant outlier cells; normal cells and normal-like scores are represented in yellow, all other colours represent departures from the normal-like score of subgraph activity

(2018), identification of cancer cells can be challenging and time-consuming, even for the expert eye. Deep learning architectures in general, and convolutional neural networks in particular, have proven capacity to outperform (ST4) decades-trained pathologists within the remit of cancer histopathological image classification (Supplementary Information S8).

Potentiality of deep learning is not limited to assessment of clinical images and has been leveraged for automated recognition and labelling of interior cell features, extending to identification of cell features to which the in silico system was formerly na¡ve (Ounkomol *et al.*, 2018; Supplementary Information S9). Indeed, as an exemplary market disruptor, Deep Variant translates genomic information into image-like representations which are then amenable to analysis as images. Deep Variant represents a wider principle; the methodological approach of applying deep-learning algorithms to visual abstractions represents a viable competitor to conventional approaches. Ultimately, deep learning offers the opportunity to outpace evolution of resistance to combinatorial targeted therapies, delineated in the concluding section of this review.

5 Outpacing resistance

Computational biology approaches facilitate formulation of non-intuitive predictions from visually intractable data. Non-intuitive predictions are usually ominous of emergent systemic behaviours—including bistability, ultrasensitivity and robustness—that are explicable in terms of interactomics (Aebersold *et al.*, 2009; Bhalla and Iyengar, 1999). In unrelated areas of inquiry, artificial intelligence has proven capable of eliciting non-intuitive pattern recognition from high volume data (Gebru *et al.*, 2017). Therefore, deep learning architectures are the leading candidate in elucidating non-intuitive feature prediction in the subpopulation of chemoresistance-bearing outlier cells.

For the benefit of readers seeking to apply 6D phase portrait analysis, recapitulation of the methodology detailed in Kim *et al.* (2017) is recommended as a pragmatic starting point. Since Kim *et al.* (2017) focus on three dimensions of carcinogenesis, the methodology will need to be adapted to 6 dimensions, and ultimately applied to single cells. By necessity, the intended output at this stage is basic research applications of identifying resistance-bearing outlier cells. Advances in technological capacity and philosophical re-imagination of cancer will hopefully convert basic research findings into clinical adjuvants (Supplementary Information S10).

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