CANCER ORIGINS

# Tumorigenesis: it takes a village

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Abstract | Although it is widely accepted that most cancers exhibit some degree of intratumour heterogeneity, we are far from understanding the dynamics that operate among subpopulations within tumours. There is growing evidence that cancer cells behave as communities, and increasing attention is now being directed towards the cooperative behaviour of subclones that can influence disease progression. As expected, these interactions can add a greater layer of complexity to therapeutic interventions in heterogeneous tumours, often leading to a poor prognosis. In this Review, we highlight studies that demonstrate such interactions in cancer and postulate ways to overcome them with better-designed therapeutic strategies.

Clones
Groups of cells that each originate from a common ancestor and share the same set of genetic and epigenetic alterations. Any new subset of changes occurring within clones gives rise to subclones.

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*The whole is more than the sum of its parts.* Aristotle, *Metaphysics*[1]

This famous quotation from the ancient Greek philosopher Aristotle may not be applicable to all situations, but it is certainly turning out to be true in the field of oncology. Although cancer was studied as a clonal disease for many decades[2], some very early studies of mouse mammary tumours revealed that cellular subpopulations from different sections of the same tumour vary in growth rate, immunogenicity, drug response and ability to metastasize, thereby demonstrating functional and phenotypic heterogeneity[3]. It is now beyond doubt that most cancers possess considerable intratumour phenotypic heterogeneity that can be both heritable and non-heritable, arising from both genetic and non-genetic variability within the tumours[4–7]. Often, variations in the availability of resources within a tumour, such as differential access to nutrients and oxygen owing to the tumour architecture, can be a driving force that generates intratumour heterogeneity[8,9]. It is true that Darwinian forces of evolution act on heritable phenotypes and not genotypes, but because functional phenotypic variability in the tumour milieu has historically been difficult to study in patients, most studies of tumour heterogeneity have focused on genetic heterogeneity rather than on phenotypic heterogeneity that arises due to environmental selection forces in the tumour. However, despite our growing knowledge about cellular heterogeneity in cancer, we are far from understanding the dynamics that operate among the heterogeneous subpopulations and their role in disease progression and therapeutic responses.

Intratumour heterogeneity was recognized as an inherent property of many types of murine tumours as early as the 1970s. Although these findings were initially met with overwhelming criticism and disbelief from the mainstream followers of the clonal origin of cancer hypothesis, very soon tumour heterogeneity began to be accepted as a part of tumour evolution[3]. Early *in vitro* and *in vivo* studies of artificially introduced intratumour heterogeneity in murine models were able to illustrate phenomena such as variations in metastatic capacity or differences in drug sensitivity compared with tumours composed of single clones[3,10–12]. Although most observations were those of clonal interference and competition[13], some clearly indicated clonal cooperation in the form of mutualism or synergism[14,15] (BOX 1). Unfortunately, these initial demonstrations of clonal interactions went under the radar for more than two decades as research during that time mostly focused on cell-autonomous oncogenes and tumour suppressors[16].

However, as the importance of intratumour heterogeneity came into the limelight once more, the need to understand the population dynamics that operate within heterogeneous tumours began to be recognized. Thus, cancer is now commonly viewed and analysed as an evolving ecosystem[17,18]. It is expected that, just as in any ecosystem or as in the context of organs, cancer cells engage in heterotypic interactions with cells in their microenvironment and use the available resources to proliferate and survive[19]. Moreover, recent discoveries are bolstering the idea that individual subpopulations of cancer cells also behave rather like societies and substantially interact with one another, just as scientists in the field had postulated more than three decades ago[20]. Thus, it is not surprising that multiple populations that

Clonal interference
A phenomenon in which multiple clones of higher than average fitness coexist in the same population and interfere with each other. It results from negative interactions that eventually reach equilibrium. Clonal interference is thought to slow down evolution.

exist within close proximity and that compete for limited resources could inadvertently engage in many complex interactions resulting from Darwinian forces.

Although the vast majority of subclonal interactions could be neutral, the interactions that manifest phenotypically are often either negative or positive, although negative and positive interactions can also occur concurrently (BOX 1). Negative interactions can arise due to clonal competition that eventually results in selective sweeps in concordance with the 'survival of the fittest' aspect of Darwinian evolution. These kinds of interactions can occasionally lead to induced cytotoxicity of the less fit subclones[21,22], or the more-fit population can simply outcompete other subclones and take over the tumour landscape[23]. Over time this can result in clonal selections and diminished heterogeneity within tumours. Conversely, positive interactions mostly emerge as clonal cooperation and can be thought of as one of the major drivers of persistent intratumour heterogeneity. These types of collaborative interactions can be a result of mutualistic or synergistic tendencies that are intended to benefit the tumour as a whole (BOX 1). For the sake of simplicity, throughout this Review we use the term clonal cooperation to describe both mutualism and synergism without discrimination. The idea of clonal cooperation also supports the possibility that instead of one clonal population accumulating all the mutations that enable it to acquire the 'hallmarks of cancer' (REF. 24), which is undoubtedly time consuming and inefficient, several cooperating partially transformed subclones may, in theory, circumvent full transformation by benefiting one another and thus accelerating

## Box 1 | Types of ecological interactions among tumour cell populations

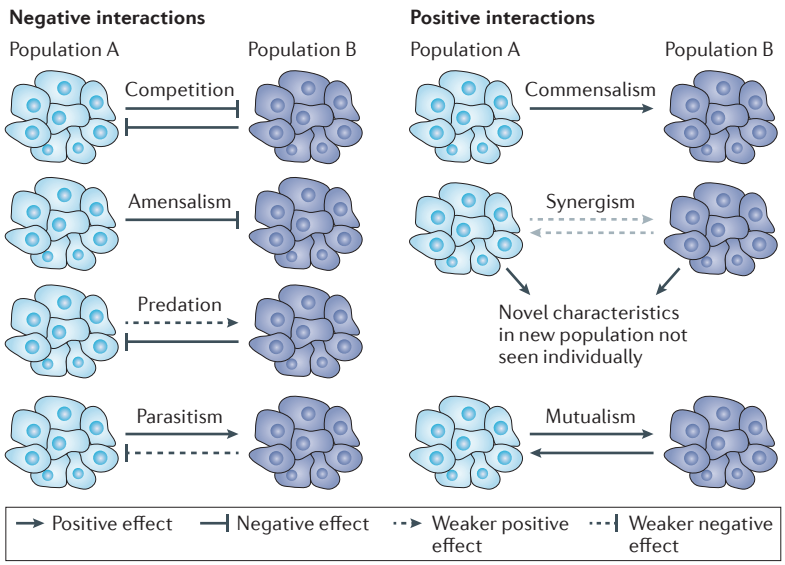
Just as is observed in an ecological habitat shared by diverse species, heterogeneous populations of cancer cells that reside in close proximity are thought to engage in a variety of interactions that may influence their fitness and survival. These interactions can be direct or can be mediated via the tumour microenvironment[18,37]. Very broadly, the interactions observed in ecological systems can be classified into two major groups (see the figure).

**Negative interactions**

The strongest negative interaction seen among neoplastic populations is competition. It usually arises due to limitations in resources such as nutrients and oxygen, and it can manifest phenotypically via the secretion of molecules by one cell population that can either kill or suppress competitor cells and vice versa. Similar to competition but occurring more unidirectionally, amensalism involves the inhibition of one population (B, see the figure) by another (A), without population A being affected in any way. These interactions can eventually result in the extinction of weaker subclones and clonal dominance of the more fit sub-clonal population. Other antagonistic relationships that are seen in ecological systems include parasitism and predation, which benefit one population by consuming biomass at the expense of the other. However, neither of these is likely to be seen among cancer cells within tumours, although cancer cell–immune cell interactions can be considered a form of predation.

**Positive interactions**

- Commensalism is a type of positive interaction by which one population can benefit another without being affected itself. Because of the diffusible nature of growth factors, signalling cytokines and shared resources distributed by the circulation, commensalism could result in the proliferation of 'free-rider' or 'cheater' subclones that take advantage of the resources contributed by the 'producer' subclones. However, if the free-riders or cheaters outcompete the producers, the shared resources are lost and the tumour may collapse.
- Synergism is another type of positive interaction by which two or more populations give rise to novel characteristics in the whole system that are absent if either population is present alone, without necessarily having an effect on the individual population (see the figure; grey arrows represent an indirect effect). Synergism endows the subclones, and also the overall tumour, with novel properties (such as the ability to metastasize[53]) that are not seen otherwise.
- Mutualism arises when two or more populations cooperate and produce factors or bring in resources that will benefit all of the interacting parties, as is evident in symbiotic relationships between two organisms in nature. In cancer, mutualism is thought to be capable of increasing the fitness of the whole tumour by enhancing the survival of the heterogeneous subclones.



'Free-rider' or 'cheater' subclones
These are cancer cell populations that take advantage of resources produced by other cancer cell populations within the tumour to proliferate and survive without any obvious reciprocation to their neighbours.

tumour progression[25]. Also of note, such collaborative interactions might indicate that the 'unit of Darwinian selection' in cancers could be oligoclonal rather than monoclonal, although this is likely to be very rare. In addition, it is important to consider that collaborative dynamics might have a very high likelihood of being exploited by 'free-rider' or 'cheater' subclones and that cooperating subclones might eventually be outcompeted by a fully transformed subclone that has all the properties of the individuals in the oligoclonal mix.

In the simplest case, clonal cooperation can arise due to each subclone secreting a particular set of diffusible factors that furthers the growth of both populations, although juxtacrine signalling (for example, through Notch and WNT) may also achieve similar purposes albeit within a shorter distance range. In addition, there can be other subclones that modulate their microenvironment in certain ways that enable the tumour to grow bigger and to metastasize. For example, in theory, one subclone could secrete angiogenic factors to enhance blood vessel formation and could thereby bring in nutrients and oxygen to facilitate growth, whereas its partner could produce proteinases that degrade the surrounding extracellular matrix to allow invasion and dissemination. When present alone, these traits are not enough to promote widespread tumour growth, metastasis and the emergence of therapeutic resistance. When present in combination, however, these traits can increase the scope of multiple populations to progress and thrive more than they could have done individually[25,26]. Thus, such collaborative crosstalk among multiple subpopulations may occur either through direct cell-to-cell communication[27] or through non-cell-autonomous paracrine effects[28,29], or it could also be an indirect outcome of microenvironmental remodelling[28,30] (FIG. 1). In extreme cases, the cancer cells themselves can directly participate in bringing in resources to the tumour by vascular mimicry[31,32]. Either way, the overall outcome is the progression of the tumour and subsequent dissemination to secondary organs, much to the disadvantage of the host organism.

Clonal cooperation can thus have considerable effects on tumorigenesis, disease progression and therapeutic outcomes. Nevertheless, even though the concept has been known for many decades, most early studies of cellular cooperation were purely observational and lacked mechanistic insights owing to the absence of proper tools[20]. Direct experimental demonstrations and mechanistic dissection of such cooperative interactions in cancers are novel in comparison to studies of clonal competition. This Review is not meant to serve as a comprehensive overview of intratumour heterogeneity. Rather, we focus on a subset of collaborative interactions that may be at play in heterogeneous tumours. Thus, by surveying examples from various model systems, we explore how cooperative interactions can influence many aspects of cancer to endow the tumour with collective and often novel characteristics. Through our explorations, we examine how the combination of changes in the tumour that are brought about by several coexisting cancer cell populations might contribute to an overall increased robustness of the whole tumour.

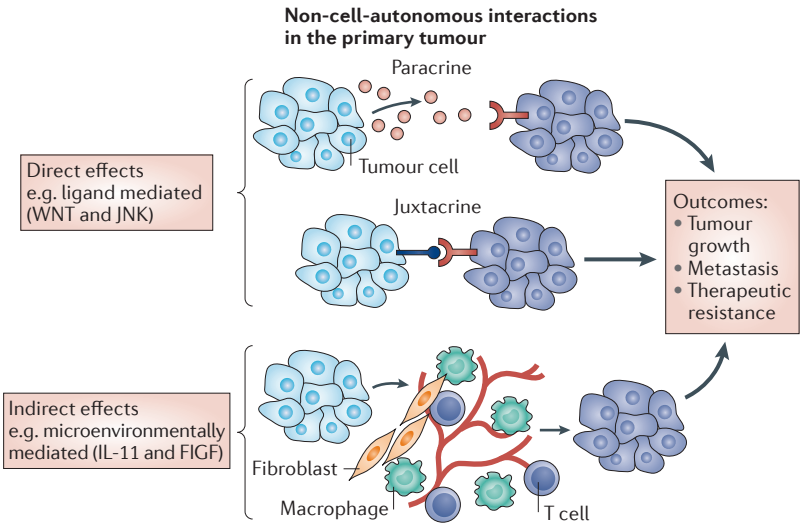



Figure 1 | **Non-cell-autonomous interactions between populations can affect tumorigenesis, metastasis and therapeutic resistance.** Non-cell-autonomous interactions may contribute to increasing the robustness of the tumour, leading to increased tumour growth, enhanced metastasis and the emergence of resistance. As exemplified by two distinct cellular populations communicating in a unidirectional manner, such interactions may occur directly through paracrine[27–29] or juxtacrine effects of ligands[49–52,54] that are produced by one cell and received by the second, or these interactions could also be indirectly mediated via components of the microenvironment, such as blood vessels, immune cells and fibroblasts[28,30,49–51]. FIGF, c-fos induced growth factor; IL-11, interleukin-11; JNK, JUN N-terminal kinase.

## Theoretical and mathematical modelling

Inter-species interactions have been extensively studied in ecology, microbiology and evolutionary biology ever since the conception of these fields. However, given the extent of heterogeneity identified in many types of cancers, it is not surprising that many of the prevalent theories that look at communication between diverse species are now also being applied to study the behaviour of subpopulations within tumours. In addition to the more traditional models that explore clonal interactions strictly from the competitive angle[33], a more contemporary view includes the acknowledgement of cooperation among subclones in the manner of mutualism or synergism. In lieu of proper tools to investigate these relationships directly in patient samples, theoretical and mathematical models have built an excellent framework to dissect the dynamics that operate among heterogeneous subclones.

A branch of theories, known as Hamiltonian medicine[34], describes many aspects of cooperative and conflicting interactions among cancer cells by closely comparing them to the dynamics of microbial interactions. Although some of this theoretical knowledge has been extrapolated from a broad range of experimental studies of bacterial interactions, other knowledge has been inferred from mathematical modelling. These theories combine basic social-behavioural perspectives with evolution to speculate how cancer cells and microorganisms alike can participate in behaviour that increases their prevalence in the host. By describing a phenomenon known as 'inclusive fitness', which is exemplified by biofilm-producing bacteria, they illustrate how

Eye-imaginal discs
A zone of cells in the *Drosophila melanogaster* larvae that give rise to the structures of compound eyes in the adult fly.

the virulence of microorganisms can be higher if many closely related species aid each other's survival. This could also very possibly be true for enhanced tumorigenicity in heterogeneous cancer cell populations[35,36].

It is widely known that cancer cells, just like bacteria, are able to secrete factors that kill nearby competitors. Theoretically, this should eventually result in the homogenization of the tumour landscape. However, such a lack of biodiversity poses a very high risk of extinction if, for example, the environment changes and, subsequently, the selective pressures change. Thus, it is understandable why maintaining population heterogeneity is beneficial[37,38]. This exact phenomenon has been illustrated particularly well in simulated studies of microbial biodiversity. One such study demonstrated that, in the absence of extreme selective pressure from the environment, the only way by which bacterial populations maintain a high degree of species diversity is by adopting a model of 'cyclic dominance'. In brief, the cyclic dominance model suggests that various smaller subgroups are maintained, in either time or space, and that they alternate in the dominance of either the sensitive, killer or resistant strains. Such coexistence is only possible because the production of a biotoxin, such as an antibiotic, has a metabolic cost to the producer, which is in this case the killer strain[39]. This phenomenon could also explain why coexisting bacterial populations may have a cumulative resistance to many antibiotics while only producing a few antibiotics themselves. Similar observations have been made in cell culture models of insulinoma, a neuroendocrine pancreatic cancer, and further verified by mathematical simulation, in which producer and non-producer subclones of a diffusible secreted factor (insulin-like growth factor 2 (IGF2)) were shown to exist in equilibrium in order to maximize their proliferative capacity[38]. Such dynamics make it possible for free-rider or cheater subclones to be maintained in tumours. However, some of these examples have already been clearly elucidated in prior reports of clonal interactions, so we do not delve into them further here[36,38,40,41].

Theoretical modelling exercises in cancer studies can be very insightful because they can simulate environmental effects that are impossible to reproduce in a biological system, such as the addition or withdrawal of a growth factor or stress[38]. They also have the unique advantage of being scalable to time frames that are not feasible in *in vivo* systems[28]. Even so, the hurdle of translating these findings into actionable therapy in humans remains, as the parameters extrapolated from simplified experimental systems may not be perfectly reproducible in patients. Furthermore, in order to reduce the complexity of these models, many assumptions are often made, which can sometimes obscure what is really happening in the tumour. Therefore, the focus is currently on developing more sophisticated technologies to obtain experimental data in clinical samples and *in vivo* animal models to better parameterize theoretical models. The ultimate goal is then to use these improved *in silico* models, in turn, to predict the behaviour of tumours over time to aid the design of more-effective therapies.

## Clonal cooperation in *Drosophila melanogaster*

For many years, *Drosophila melanogaster* has been used as a model to study negative cellular interactions, especially in the context of development. In particular, the phenomenon of cell competition among cells with differential fitness has been very well characterized in developing eye-imaginal discs[42]. Over the years, numerous studies conducted in *D. melanogaster* to elucidate the mechanisms of these short-range communications have identified several main factors involved in generating 'super-competitor' clones that are able to eradicate their neighbours[43]. In the case of *D. melanogaster* models, the factors conferring super-competitor phenotypes have been identified to be predominantly genetic, perhaps attributable to the nature of the experimental design, and include an imbalance in *dm* dosage (that is, the gene that encodes Myc); haploinsufficiency in ribosomal protein genes; mutations in tumour suppressor pathways; and the deregulation of the Hippo pathway[44–46]. Even in the absence of super-competitors, *D. melanogaster* has evolved effective mechanisms to prolong lifespan by eliminating 'unfit' but otherwise viable populations during development and ageing[47]. Studies such as these have inspired researchers to investigate cell competition and other similar cellular interactions in mammalian systems as well[48]. Despite being functionally different, these and other similar studies have also provided important insights into some of the mechanisms that may be at play among interacting cancer cells.

In conjunction with cell competition studies, the genetic tools available in *D. melanogaster* have also made it a highly amenable model for simple but illustrative studies of oncogenic cooperation *in vivo*. Many remarkable studies utilizing *D. melanogaster* genetics examined how cooperative interactions can alter tumour behaviour. For example, several studies demonstrated that interactions between cells with oncogenic $Ras^{V12}$ mutations and adjacent cells lacking the gene that encodes scribbled ($scrib^{-/-}$) can promote widespread overgrowth and invasion of the $Ras^{V12}$-mutant cells in the *D. melanogaster* eye-imaginal discs while the $scrib^{-/-}$ population is maintained as a minor subclone[49–51]. However, more remarkable is the complete absence of such a profound behaviour if all the cells express $Ras^{V12}$. The mechanism of cooperation was found to be JUN N-terminal kinase (JNK) activation by $scrib^{-/-}$ clones both cell autonomously and non-cell autonomously, leading to the induction of stress-induced JNK activity in $Ras^{V12}$-expressing cells and the upregulation of cytokines activating Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling. Interestingly, if the $scrib^{-/-}$ cells were eliminated or outcompeted from the system, the added benefit to the $Ras^{V12}$ cells was lost[51]. However, tissue damage (for example, mechanical wounding) was able to induce the proliferation of $Ras^{V12}$ cells even in the absence of $scrib^{-/-}$ clones, implying that compensatory proliferation is the underlying mechanism.

In a similar but slightly different system also using *D. melanogaster* eye-imaginal discs, another group[52] demonstrated that, in a $Ras^{V12}$-driven tumorigenesis

model, a minor population of cells harbouring mitochondrial dysfunction (*Pdsw*$^{-/-}$ (which encodes a subunit of NADH dehydrogenase) or cytochrome *c* oxidase subunit 5a (*CoVa*$^{-/-}$)) can influence adjacent cells with normal mitochondrial function in a juxtacrine manner, leading to oxidative stress and the metastasis of the *Ras*$^{V12}$ cells also via JNK-mediated activation of the Hippo pathway[52]. Once again similar to the cell competition studies, by virtue of the robustness and simplicity of *D. melanogaster* models, these mechanistic findings of clonal cooperation can help us to identify some of the key pathways that may be operating in human tumours.

### Clonal crosstalk in mouse models

With the advancement of molecular biology techniques over the past few decades, more thorough studies that illustrate subclonal interactions have been conducted in animal models, shedding more light on to the early observations of subclonal cooperation in heterogeneous tumours by cancer biologists in the 1980s and 1990s[14,15]. In one such example from recent years, Calbo and colleagues[53] used a genetically engineered mouse model of small-cell lung cancer to describe that, when present together, two distinct populations of cancer cells transplanted together can lead to disease progression and liver metastasis, but that they are unable to do so when transplanted individually. One of the interacting populations was phenotypically neuroendocrine, and it was able to induce the proliferation of a less differentiated non-neuroendocrine population *in vitro*. The results *in vivo* were even more remarkable in that the non-neuroendocrine population was able to establish metastatic lesions in the liver only when mixed with the neuroendocrine population. Akin to cases seen in *D. melanogaster*, this mouse model illustrated how the presence of two different populations benefited tumour progression. However, in contrast to the *D. melanogaster* model, in which the two interacting populations evolved separately[49–51], the non-neuroendocrine cells in the mouse model were shown to arise from the neuroendocrine population owing to the loss of differentiation markers induced by the activation of RAS signalling. This mouse example exhibited the phenomenon of one population endowing the other with metastatic potential. The other noteworthy observation from this example is that the non-neuroendocrine population was only present as a minor population in the primary tumours even though it dominated the metastatic lesions. One can imagine the immense layer of complexity that these interactions can add to the design of treatment strategies, and such phenomena could potentially explain the lack of response of metastatic disease to therapies designed to target the dominant subclones that are present in the primary tumour (FIG. 2).

A similar but slightly different mode of clonal cooperation was observed in a mouse model of breast cancer that was driven by mouse mammary tumour virus (MMTV)–*Wnt1* (REF. 27). In this model, two hierarchically differing subclonal populations cooperated to drive tumorigenesis through a 'division of labour'. Using somatic mutations in *Hras* (*Hras*$^{mut}$) as a marker for lineage tracing and cells from transgenic mice expressing fluorescent markers, Cleary and colleagues[27] demonstrated cooperative interactions between basal *Hras*$^{mut}$*Wnt1*$^{low}$ subclones and luminal *Hras*$^{wild\text{-}type}$*Wnt1*$^{hi}$ subclones. Both populations were required for tumorigenesis but it was heavily dependent on WNT1 production by the luminal subclones. In order to examine this cooperative behaviour more closely, the authors transplanted mammary epithelial cells that consisted of basal populations from closely related transgenic animals that harboured doxycycline-inducible *Wnt1* into animals that were either wild type or that constitutively expressed *Wnt1*. Interestingly, they found that, upon doxycycline withdrawal, the tumours in wild-type animals regressed almost completely, although some residual cells remained that could cause tumour regrowth after re-administration of doxycycline. By contrast, the tumours in the *Wnt1*-expressing mice only partially regressed before recurring even in the absence of *Wnt1* induction. More surprising was the observation that the recurring tumours of *Wnt1*-expressing mice had a large number of luminal cells from the recipient mouse in the tumours, which further reinforced the idea of

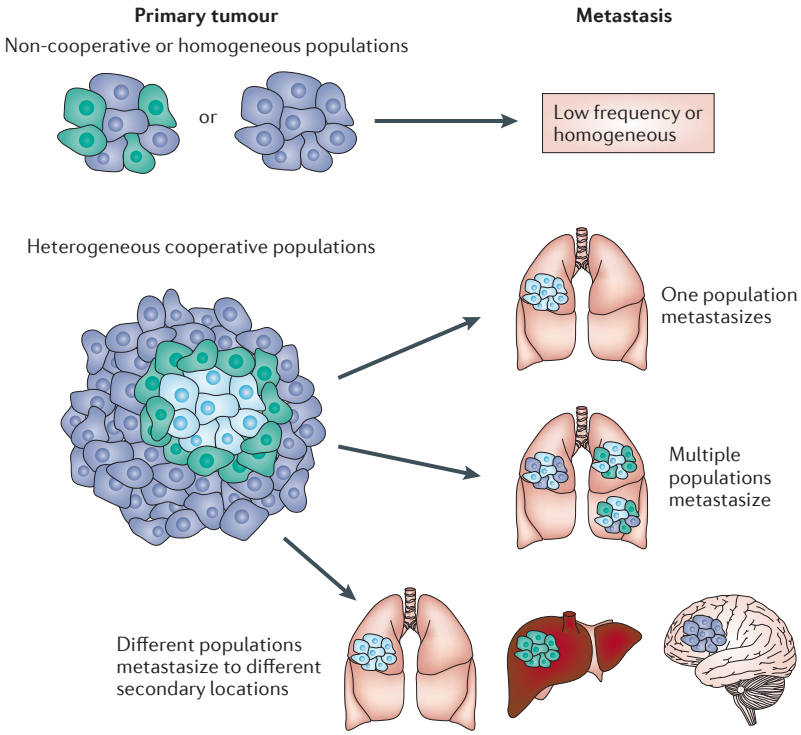



Figure 2 | **Unique properties of heterogeneous tumours using gain of metastatic potential as an example.** Homogeneous primary tumours or those composed of non-cooperative populations may have a limited ability to form metastases in distant organs (top). By contrast, the metastatic potential of heterogeneous primary tumours composed of cooperative cancer cell populations (bottom) can be altered in various ways, especially if one of the clones (light blue) is a non-cell-autonomous metastasis promoter that increases the metastatic potential of neighbouring populations[28,53]. The resulting metastatic lesions may be monoclonal or polyclonal. Hypothetically, clonal cooperation may also alter the metastatic potential of individual populations such that each of them colonizes different secondary organs.

the dependence of the basal *Hras*$^{mut}$*Wnt*$^{low}$ subpopulation on cooperation from the neighbouring luminal cells to form and maintain tumours[27].

In another elegant study using a syngeneic p53-null mouse model of breast cancer, Zhang and colleagues[54] demonstrated crosstalk between CD29$^{hi}$CD24$^{hi}$ bi-potent tumour-initiating cells and a more differentiated CD29$^{hi}$CD24$^{low}$ mesenchymal population. The CD29$^{hi}$CD24$^{low}$ population was derived from the CD29$^{hi}$CD24$^{hi}$ cells. The CD29$^{hi}$CD24$^{low}$ population secreted various ligands, including WNT9A, WNT2, interleukin-6 (IL-6) and chemokine (C-X-C motif) ligand 12 (CXCL12), and the CD29$^{hi}$CD24$^{hi}$ population showed gene expression changes in response to some of these factors and expressed high levels of some of the corresponding receptors such as CXCR4 (CXCL12 receptor), thereby indicating crosstalk between the two populations. Furthermore, the authors showed that cytokines secreted by CD29$^{hi}$CD24$^{low}$ cells stimulated the self-renewal and tumour-initiating capacity of the CD29$^{hi}$CD24$^{hi}$ cells, thus establishing a positive feedback loop. This study was an outstanding example of interactions between cancer cells with more stem cell-like phenotypes and their more differentiated derivatives similar to that observed in experimental models of prostate cancer[29]. Although one might argue that the interactions depicted in this model and the ones mentioned above are over-simplified and not completely representative of those occurring in the breast tumours of patients, the clear illustration of cooperative dependence of two distinct subpopulations on each other for successful tumorigenesis is still valuable for our overall understanding of these interactions in cancers[27,54].

## Cooperation of human cancer cells

Xenotransplantation assays using human cells have been used to study different aspects of tissue development and tumour progression for many years, but more recently they have been used to ask specific questions regarding clonal interactions in cancer. Even though these models may not be perfect representations of what happens in patients, they have the unique advantage of being amenable to molecular manipulation. Several such studies conducted over the past few years have provided important insights into mechanisms that maintain heterogeneity and clonal cooperation within the tumours and how these can affect tumour behaviour. Although most of these studies have used simplistic approaches to look at interactions between two or a limited set of distinct subclones to reduce complexity, the general observations can be extended to more intricate systems. For example, in a model of human melanoma cell lines xenografted in zebrafish, Chapman and colleagues[30] were able to show how interactions between diverse subclones allow for reversible phenotype switching of the participating clones. More precisely, they identified that a poorly invasive subclone secreted extracellular matrix components only in the presence of an invasive subclone, which induced the invasive subclone to switch from a protease-independent mode of invasion to a membrane-type matrix metalloproteinase (MT1-MMP)-dependent phenotype, thereby promoting the invasion of both populations. In another model using the PC-3 prostate cancer cell line *in vitro* and in mice, Mateo and colleagues[29] showed that a non-cancer stem cell (non-CSC)-like population could increase the invasiveness of a CSC-enriched population by secreting SPARC, an extracellular matrix protein[29]. An additional point to make is that data from many studies focusing only on CSCs as a cause of therapeutic failure have not been fully supported empirically[55], indicating that some kind of clonal interaction could potentially be at play between CSCs and non-CSCs in many such cases, as we have seen in the example above. Thus, even simple xenograft studies like the ones mentioned above are beginning to add experimental evidence to support the theory of mutually beneficial communication between heterogeneous populations.

In order to explore beyond bi-clonal dynamics, our laboratory took xenograft modelling a step further by deriving approximately 20 different subclones via lentiviral overexpression of cancer-promoting factors in a triple-negative breast cancer cell line, MDA-MB-468, and using them to interrogate tumour behaviour[28]. By comparing tumour growth in the context of each subclone against the parental cell line (monoclonal tumours) versus growth in the tumours in which all the clones were present in equal initial frequencies (polyclonal tumours), we discovered that polyclonal tumours were much more aggressive (larger and more metastatic) than monoclonal tumours. Using a reductionist approach, we were able to reproduce metastatic behaviour using only two cooperating subclones — subclones overexpressing IL-11 and c-fos induced growth factor (FIGF; also known as VEGFD) — although it is possible that some of the other subclones may also have had some influence on the overall tumour. In our model, the IL-11-overexpressing clone acted as a non-cell-autonomous driver of tumour growth as it constituted only a small proportion of the final tumour, while the FIGF-overexpressing subclone may have taken advantage of the growth promotion to reach sufficient numbers to allow increased lymphangiogenesis (promoted by FIGF[56]) and subsequent dissemination of the tumour cells. Furthermore, by extrapolating our observations using mathematical modelling, we also showed that the presence of IL-11 and clonal interactions were required to maintain the overall heterogeneity of the tumour owing to non-cell-autonomous driving of tumour growth by IL-11 and clonal interference. This is a situation in which multiple clones of higher than average fitness coexist in the same population and interfere with each other to prevent any of them from taking over the tumour population. Our results indicated that when the IL-11-overexpressing subclone was present, all the subclones maintained equilibrium, but when the IL-11-overexpressing subclone was removed, the tumour either failed to grow or suffered necrotic collapse[28]. Similar findings were also reported in glioblastoma models in which minor subpopulations

Phenotype switching
The ability of cells to change their phenotype in response to the environment. It is usually a result of changes in epigenetic modifications within the cell and is reversible.

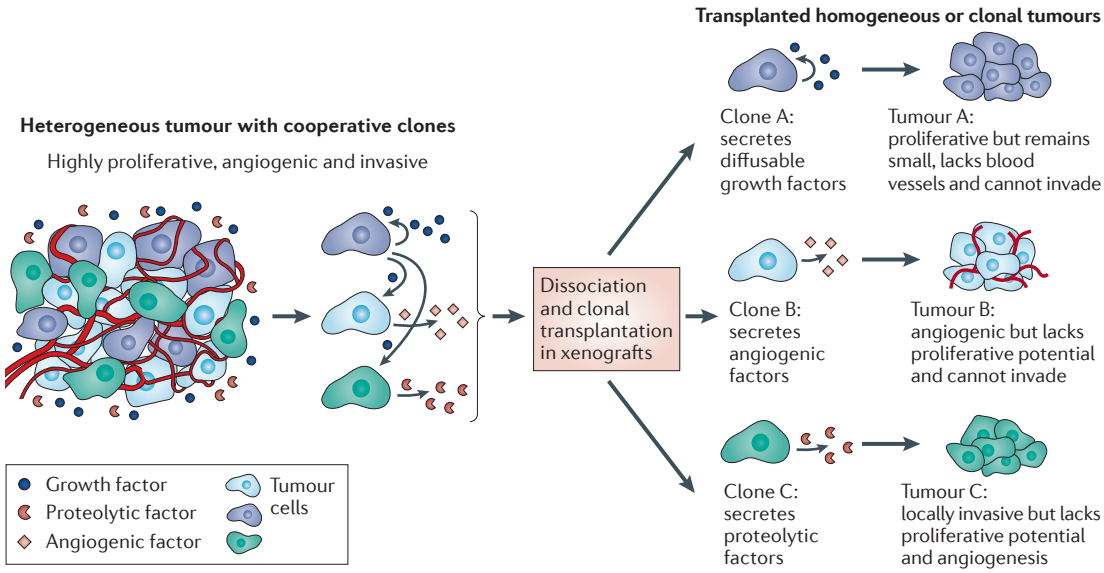



Figure 3 | **Deficiencies of xenograft assays using homogeneous cell populations or single cells.** Monoclonal or single-cell transplantation assays may not reflect the behaviour of cells in the intact polyclonal heterogeneous tumours from which they are derived because of the lack of cellular interactions. A heterogeneous tumour has various subpopulations engaged in a network of ecological interactions (BOX 1). In a perfectly cooperative situation, it is possible that different subclones contribute different factors to aid the overall growth of the tumours. However, many xenograft assays rely on single-cell transplantation methods that eradicate the opportunity for interclonal interactions that were present in the original tumour. Thus, these monoclonal tumours will fail to reproduce the behaviour of the parental tumour. In addition, non-cell-autonomous interactions mediated via the microenvironment may not reproduce well owing to species differences.

expressing mutant epidermal growth factor receptor (EGFR) were shown to be responsible for maintaining tumour heterogeneity[57].

In addition to cell lines and engineered clones, patient-derived xenograft (PDX) models have also demonstrated the maintenance of heterogeneity with minimal clonal selection across multiple passages. Several such studies have used barcoded lentiviral transduction systems to track clonal distribution in the xenografts and compared them with the original patient tumour to show that polyclonality and high levels of sub-clonal heterogeneity can affect tumour behaviour differently from more homogeneous engraftments[58]. Although the evidence for sub-clonal cooperation is not completely conclusive from observations in these PDX studies, the general preference for maintaining high levels of intratumour heterogeneity definitely suggests that this has some evolutionary advantage for the tumour[32,58,59]. Studies like these also demonstrate why PDXs that are derived from the transplantation of single cells or homogeneous populations may not represent the true dynamics that operate in the original heterogeneous primary tumour in the patient. Such homogeneous populations are essentially devoid of the interclonal interactions that might be important in dictating the behaviour of the tumour (FIG. 3). However, despite the relative ease of detailed clonal tracking that is available with modern technologies, gaining mechanistic insights with PDX models is still not as straightforward as with engineered cell line models, thus resulting in a preference for cell line models in many cases.

Recovery periods and drug holidays
As most therapeutic regimens have some accompanying side effects, patients are usually taken off the treatment for short intervals to allow for recovery from systemic toxicity. Sometimes drug holidays are also scheduled to increase the efficacy of the treatment.

## Clonal cooperation and cancer therapy

There is rapidly accumulating evidence that a high degree of heterogeneity can imply poor disease prognosis in patients[60–62]. Recent studies have shown that metastatic breast cancer lesions are more likely to arise from oligoclonal circulating tumour cell (CTC) clusters, which again points to the benefit of clonal interactions[63]. Thus, it is expected that intratumour heterogeneity may affect therapeutic outcomes. Furthermore, clonal dynamics can change in response to therapy to establish new heterogeneous populations that may confer resistance to treatment[59]. This often happens during recovery periods and drug holidays. These scenarios are comparable to observations of rapid evolutionary changes in nature; for example, after the particularly strong El Niño, which is a relatively rare climactic event, in the early 1980s that drastically modified the food supply, Galapagos finches had to quickly adapt to the altered environment in order to maximize their survival in the region[64].

A handful of studies in highly heterogeneous cancers such as glioblastoma have alluded to the presence of multiple coexisting subpopulations, each with unique mutations that could be independent (spatially or temporally) drivers of tumorigenesis. In the case of glioblastoma, amplification of three different receptor tyrosine kinases with known oncogenic roles — EGFR, MET and platelet-derived growth factor receptor (PDGFR) — was observed in three individual cancer cells within a single tumour from a patient, implying the coexistence of at least three independent clones[65].

More recently, single-nucleus sequencing has revealed the presence of distinct EGFR-truncating alterations (EGFRvII and EGFR carboxy-terminal deletions) in non-overlapping subclones that are thought to have evolved independently[66]. Although EGFRvII has been shown to be oncogenic and sensitive to EGFR inhibitors, little is known about the EGFR C-terminal deletion mutants. The current hypothesis is that these populations that have various EGFR alterations, in the presence of other accumulated mutations, could be one of the reasons for resistance to targeted therapy in glioblastoma.

Although studies that show the presence of multiple drivers in patient tumours do not directly prove clonal interactions, they enable us to infer that there might be some interplay among different driver subclones that contributes to resistance by changing sub-clonal compositions when a tumour is subjected to therapeutic intervention. For example, cellular diversity scoring of breast tumour samples before and after neoadjuvant chemotherapy using immunofluorescence and fluorescence *in situ* hybridization (iFISH) analysis has revealed very little change of the cellular genetic diversity in partially responding and non-responding tumours, although the individual populations constituting the tumour were phenotypically different[62]. Such studies provide us with indirect evidence that maintenance of intratumour heterogeneity (perhaps not by the same initial subpopulations, but by similar ones nonetheless) is most likely to be the way in which most cancers evade therapy and lead to disease relapse in patients.

It is well known that the presence of resistant subclones within the tumour milieu that are undetectable at diagnosis using commonly applied methods can contribute to eventual treatment failure and relapse[67–69]. For example, oestrogen receptor-α (*ESR1*) mutations have not been detected in primary breast tumours but a significant proportion of metastatic lesions that are refractory to endocrine therapy contain *ESR1*-mutant cells[69–73]. However, with further technological advances enabling the detection of single or rare mutant cells, such as digital PCR[74], some of these pre-existing mutants that are currently eluding diagnostic testing may become visible. Although quite simplified, some *in vitro* work using co-culture systems has already demonstrated that a small subpopulation of resistant clones could aid the outgrowth of non-resistant clones[75], and similar interactions may also be applicable in the tumours of patients. Thus, one can imagine the challenges that therapeutic interventions may face if several sub-clonal populations aid each other's survival and/or dissemination in a similar manner, making it even more difficult to eradicate the disease. More daunting is the emerging evidence of small populations of malignant cells recruiting diverse populations of benign cells and transforming them to enhance tumour growth either by direct stimulation, such as the secretion of activating cytokines[76] and promoting the loss of tumour suppressors[77], or by indirect mechanisms, such as exosomal delivery of RNA or protein factors[78]. This kind of recruitment, although slightly different from clonal cooperation, indicates that successful tumour formation often relies on the contribution of more than one subpopulation, even if only to increase the sheer size and spread of the tumour. Unfortunately, these corruptive influences are still poorly understood, and we know even less about how to eliminate these 'influential' clones.

Another hurdle in the path of cancer therapy is the emergence of transient cooperating clones. In studies of bacterial antibiotic resistance, one term that is frequently encountered is 'persistence'. Not to be confused with 'resistance', persistence implies the ability of otherwise sensitive bacterial strains to withstand an onslaught of antibiotic attack by slowing down growth and metabolism, almost to a state of dormancy (BOX 2). Additionally, these persister populations could arise from any bacterial cell without requiring a particular mutation to evade the antibiotic[79]. A similar programme has been described in tumours, in which a subpopulation can reversibly and transiently alter its epigenetic profile via engagement of IGF1 receptor (IGF1R) activity to form a drug-tolerant persister population[80]. Once the therapy is removed, the surviving quiescent persisters are then able to revert to more proliferative tumorigenic populations. Therefore, tumour heterogeneity — be it transient or lasting, heritable or non-heritable — represents a major obstacle towards understanding and treating cancers.

Cellular diversity scoring
Widely used in ecology, diversity scoring is a process of quantifying heterogeneity in an environment by taking into account the number and abundance of its inhabiting species. In tumours, the cellular diversity score is a number that represents the extent of unique sub-clonal populations that contribute to the intratumour heterogeneity.

Box 2 | **Mechanisms underlying drug insensitivity**

Similar to the mechanisms that are responsible for antibiotic resistance in bacteria, cancer cells can evade therapy in several ways:

***Resistance.*** Resistance is a state of a lack of response to the administered therapy and can be either intrinsic or acquired. Intrinsic resistance could result from the presence of a subset of cells either that do not possess a receptor for the particular drug or that do not rely on the targeted pathway for growth and survival. By contrast, acquired resistance can be the result of a new mutation that alters the binding of the drug to its target binding site, amplification or overexpression of the target, or the activation of compensatory signalling pathways to the one being inhibited. Although resistant states are mostly attributable to genetic changes, heritable epigenetic alterations have also been shown to be responsible for drug insensitivity[89,90].

***Persistence.*** Unlike resistance, persistence is a reversible trait that can be exhibited by any cell in the tumour population without requiring any new mutations. An example of this is when a small population of cells enters a state of quiescence by drastically decreasing the activity of their metabolic pathways. Persistence is a reversible state that is thought to be regulated epigenetically[79].

***Drug tolerance.*** This is a phenomenon in which cells gradually become non-responsive to a drug over time such that increasing dosages are required to achieve the same effect[80]. At some point, systemic toxicities become dose limiting and the drug can no longer be used effectively for treatment.

## Concluding remarks and future directions

Although communication among cells is an essential part of embryonic development and tissue homeostasis, this phenomenon was vastly underappreciated in the realm of cancer biology until very recently. Interactions between heterogeneous cell populations were noticed by cancer biologists very early on, but the lack of general

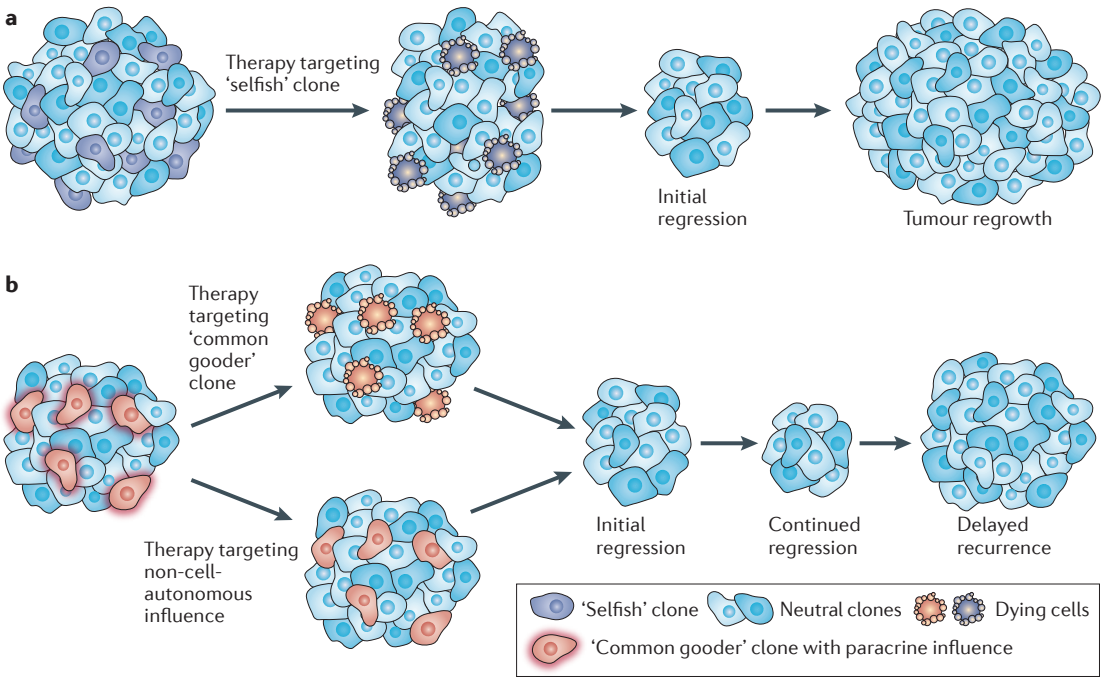



Figure 4 | **Improving therapeutic design for heterogeneous tumours.** **a** | Targeted therapies can fail to prevent tumour growth if the targeted subclone is 'selfish' or has no influence on the behaviour of other populations within the tumour. Even if there is an initial reduction in tumour size, the other neutral populations that are non-responsive to therapy can keep growing and maintain the tumour, leading to relapse. **b** | Better understanding cooperative interactions may help us to identify the non-cell-autonomous drivers or 'common gooder' subclones that promote tumour growth by influencing nearby populations. If therapeutic interventions can eliminate these populations or mute their paracrine stimuli, the overall growth of the tumour could be stopped. At the very least, even if resistant populations eventually emerge, tumour relapse would be delayed under these circumstances.

interest, as well as proper tools, prevented their thorough mechanistic investigation for many years. In this Review, we have discussed many of the emerging studies of clonal interactions in various model systems of cancer, specifically focusing on cooperative behaviours that can influence tumour progression.

It is clear that clonal cooperation can have a profound effect on therapeutic outcomes in cancer, and it can also make the design of targeted therapies very challenging. As exemplified in microbial systems, cooperation may lead to interdependence and comes at both a metabolic and an evolutionary cost[81], however, the benefits of maintaining intratumour heterogeneity must outweigh the expense[37]. Thus, our focus must be veered towards a more ecological therapy of cancers by which mechanisms of clonal cooperation are identified and their modes of interaction are targeted rather than aiming for the elimination of all individual subclones[17]. Some researchers also believe in shifting the balance of the cost–benefit ratio of intratumour heterogeneity and preventing recurrence by using treatment strategies to stimulate benign (or less altruistic) subclones to outgrow the potentially resistant subclones before therapeutically attacking the whole tumour[26].

Another approach along these lines would be to identify the 'common gooders' and eliminate them in a targeted manner, to eradicate the paracrine effectors, or to remove the 'public goods' upon which all the populations rely[38,82–84]. If the general tumour populations are not dependent on the targetable population, eliminating the targetable cells has no effect on overall tumour growth (FIG. 4a). However, if the neutral clones do depend on the targetable population, perhaps owing to some paracrine effect of diffusible products, eradicating that population or blocking the factor itself could lead to tumour reduction[85] (FIG. 4b). Recent technological advances have been enabling the long-term and periodic monitoring of some cancer types via non-invasive liquid biopsies that analyse biomarkers, CTCs or circulating tumour DNA (ctDNA) in the blood[86–88]. As sample collection and analysis techniques continue to improve, such data will be able to give insights into the heterogeneity of the primary tumour and its metastases and how these may change over time during treatment. It will be very beneficial if we can exploit such methodologies to decipher additional information about sub-clonal interactions in order to more comprehensively understand a tumour. Although more knowledge about tumour ecosystems is accumulating, it is becoming increasingly clear that we will only be able to effectively eliminate the ever-evolving cancer populations and achieve lasting cures in patients if our therapeutic strategies incorporate intratumour heterogeneity into their design.

Common gooders
Populations of cells that can act as non-cell-autonomous drivers of tumour growth through the secretion of diffusible factors that can have positive paracrine influences on neighbouring populations.

Public goods
A term from the field of economics that denotes resources that are consumed by the entire society rather than an individual. In the tumour milieu, public goods can be exemplified by diffusible growth factors, nutrients and oxygen.

1. Aristotle *Metaphysica* Vol. 8, 1045a (1933).
2. Nowell, P. C. The clonal evolution of tumor cell populations. *Science* **194**, 23–28 (1976).
3. Heppner, G. H. Tumor heterogeneity. *Cancer Res.* **44**, 2259–2265 (1984).
4. Marusyk, A., Almendro, V. & Polyak, K. Intra-tumour heterogeneity: a looking glass for cancer? *Nat. Rev. Cancer* **12**, 323–334 (2012).
5. Gerlinger, M. *et al.* Cancer: evolution within a lifetime. *Annu. Rev. Genet.* **48**, 215–236 (2014).
6. McGranahan, N. & Swanton, C. Biological and therapeutic impact of intratumor heterogeneity in cancer evolution. *Cancer Cell* **27**, 15–26 (2015).
7. Kreso, A. & Dick, J. E. Evolution of the cancer stem cell model. *Cell Stem Cell* **14**, 275–291 (2014).
8. Lajara, R. *et al.* Dual regulation of insulin-like growth factor I expression during renal hypertrophy. *Am. J. Physiol.* **257**, F252–F261 (1989).
9. Gillies, R. J., Verduzco, D. & Gatenby, R. A. Evolutionary dynamics of carcinogenesis and why targeted therapy does not work. *Nat. Rev. Cancer* **12**, 487–493 (2012).
10. Fidler, I. J. & Kripke, M. L. Metastasis results from preexisting variant cells within a malignant tumor. *Science* **197**, 893–895 (1977).
11. Dexter, D. L. *et al.* Heterogeneity of tumor cells from a single mouse mammary tumor. *Cancer Res.* **38**, 3174–3181 (1978).
12. Fidler, I. J. Tumor heterogeneity and the biology of cancer invasion and metastasis. *Cancer Res.* **38**, 2651–2660 (1978).
13. Miller, B. E., Miller, F. R., Wilburn, D. & Heppner, G. H. Dominance of a tumor subpopulation line in mixed heterogeneous mouse mammary tumors. *Cancer Res.* **48**, 5747–5753 (1988).
**This paper was one of the first reports of mutualism between two populations of cancer cells.**
14. Miller, B. E., Miller, F. R. & Heppner, G. H. Interactions between tumor subpopulations affecting their sensitivity to the antineoplastic agents cyclophosphamide and methotrexate. *Cancer Res.* **41**, 4378–4381 (1981).
15. Miller, B. E., Machemer, T., Lehotan, M. & Heppner, G. H. Tumor subpopulation interactions affecting melphalan sensitivity in palpable mouse mammary tumors. *Cancer Res.* **51**, 4378–4387 (1991).
16. Vogelstein, B. & Kinzler, K. W. Cancer genes and the pathways they control. *Nat. Med.* **10**, 789–799 (2004).
17. Gatenby, R. A., Cunningham, J. J. & Brown, J. S. Evolutionary triage governs fitness in driver and passenger mutations and suggests targeting never mutations. *Nat. Commun.* **5**, 5499 (2014).
18. Merlo, L. M., Pepper, J. W., Reid, B. J. & Maley, C. C. Cancer as an evolutionary and ecological process. *Nat. Rev. Cancer* **6**, 924–935 (2006).
19. Egeblad, M., Nakasone, E. S. & Werb, Z. Tumors as organs: complex tissues that interface with the entire organism. *Dev. Cell* **18**, 884–901 (2010).
20. Heppner, G. H. Tumor cell societies. *J. Natl Cancer Inst.* **81**, 648–649 (1989).
21. Michelson, S. Facilitation of emergence of multidrug-resistant state by alteration of tumor environment: implications from competitive ecology models. *Cancer Treat. Rep.* **71**, 1093–1094 (1987).
22. Tomlinson, I. & Bodmer, W. Selection, the mutation rate and cancer: ensuring that the tail does not wag the dog. *Nat. Med.* **5**, 11–12 (1999).
23. Merlo, L. M., Kosoff, R. E., Gardiner, K. L. & Maley, C. C. An *in vitro* co-culture model of esophageal cells identifies ascorbic acid as a modulator of cell competition. *BMC Cancer* **11**, 461 (2011).
24. Hanahan, D. & Weinberg, R. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
25. Axelrod, R., Axelrod, D. E. & Pienta, K. J. Evolution of cooperation among tumor cells. *Proc. Natl Acad. Sci. USA* **103**, 13474–13479 (2006).
**This paper presented several theories and hypotheses of how cooperative interactions between cancer cells could be affecting tumour behaviour and how they could be investigated.**
26. Korolev, K. S., Xavier, J. B. & Gore, J. Turning ecology and evolution against cancer. *Nat. Rev. Cancer* **14**, 371–380 (2014).
27. Cleary, A. S., Leonard, T. L., Gestl, S. A. & Gunther, E. J. Tumour cell heterogeneity maintained by cooperating subclones in Wnt-driven mammary cancers. *Nature* **508**, 113–117 (2014).
**This paper provided evidence in a mouse model of breast cancer of how cooperation between distinct subpopulations is necessary for the maintenance of the tumour.**
28. Marusyk, A. *et al.* Non-cell-autonomous driving of tumour growth supports sub-clonal heterogeneity. *Nature* **514**, 54–58 (2014).
**This paper illustrated how a minor subpopulation can drive tumour growth and maintain intratumour clonal heterogeneity via microenvironmental modulation in a xenograft model of breast cancer.**
29. Mateo, F. *et al.* SPARC mediates metastatic cooperation between CSC and non-CSC prostate cancer cell subpopulations. *Mol. Cancer* **13**, 237 (2014).
30. Chapman, A. *et al.* Heterogeneous tumor subpopulations cooperate to drive invasion. *Cell Rep.* **8**, 688–695 (2014).
31. Hendrix, M. J., Seftor, E. A., Hess, A. R. & Seftor, R. E. Vasculogenic mimicry and tumour-cell plasticity: lessons from melanoma. *Nat. Rev. Cancer* **3**, 411–421 (2003).
32. Wagenblast, E. *et al.* A model of breast cancer heterogeneity reveals vascular mimicry as a driver of metastasis. *Nature* **520**, 358–362 (2015).
**This paper showed how certain subclones in a murine cancer model can participate in altruistic behaviour such as vascular mimicry to increase the metastatic dissemination of both themselves and their neighbouring subclones.**
33. Michelson, S., Miller, B. E., Glicksman, A. S. & Leith, J. T. Tumor micro-ecology and competitive interactions. *J. Theor. Biol.* **128**, 233–246 (1987).
34. Foster, K. R. Biomedicine. Hamiltonian medicine: why the social lives of pathogens matter. *Science* **308**, 1269–1270 (2005).
35. Lambert, G. *et al.* An analogy between the evolution of drug resistance in bacterial communities and malignant tissues. *Nat. Rev. Cancer* **11**, 375–382 (2011).
36. Crespi, B., Foster, K. & Ubeda, F. First principles of Hamiltonian medicine. *Phil. Trans. R. Soc. B* **369**, 20130366 (2014).
37. Marusyk, A. & Polyak, K. Tumor heterogeneity: causes and consequences. *Biochim. Biophys. Acta* **1805**, 105–117 (2010).
38. Archetti, M., Ferraro, D. A. & Christofori, G. Heterogeneity for IGF-II production maintained by public goods dynamics in neuroendocrine pancreatic cancer. *Proc. Natl Acad. Sci. USA* **112**, 1833–1838 (2015).
39. Czaran, T. L., Hoekstra, R. F. & Pagie, L. Chemical warfare between microbes promotes biodiversity. *Proc. Natl Acad. Sci. USA* **99**, 786–790 (2002).
40. Tomlinson, I. P. & Bodmer, W. F. Modelling the consequences of interactions between tumour cells. *Br. J. Cancer* **75**, 157–160 (1997).
41. Waite, A. J. & Shou, W. Adaptation to a new environment allows cooperators to purge cheaters stochastically. *Proc. Natl Acad. Sci. USA* **109**, 19079–19086 (2012).
42. Bondar, T. & Medzhitov, R. p53-mediated hematopoietic stem and progenitor cell competition. *Cell Stem Cell* **6**, 309–322 (2010).
43. Moreno, E. & Basler, K. dMyc transforms cells into super-competitors. *Cell* **117**, 117–129 (2004).
44. Baker, N. E. Cell competition. *Curr. Biol.* **21**, R11–R15 (2011).
45. Vincent, J. P., Fletcher, A. G. & Baena-Lopez, L. A. Mechanisms and mechanics of cell competition in epithelia. *Nat. Rev. Mol. Cell Biol.* **14**, 581–591 (2013).
46. Amoyel, M. & Bach, E. A. Cell competition: how to eliminate your neighbours. *Development* **141**, 988–1000 (2014).
47. Merino, M. M. *et al.* Elimination of unfit cells maintains tissue health and prolongs lifespan. *Cell* **160**, 461–476 (2015).
48. Alcolea, M. P. & Jones, P. H. Cell competition: winning out by losing notch. *Cell Cycle* **14**, 9–17 (2015).
49. Brumby, A. M. & Richardson, H. E. *scribble* mutants cooperate with oncogenic Ras or Notch to cause neoplastic overgrowth in *Drosophila*. *EMBO J.* **22**, 5769–5779 (2003).
50. Uhlirova, M., Jasper, H. & Bohmann, D. Non-cell-autonomous induction of tissue overgrowth by JNK/Ras cooperation in a *Drosophila* tumor model. *Proc. Natl Acad. Sci. USA* **102**, 13123–13128 (2005).
51. Wu, M., Pastor-Pareja, J. C. & Xu, T. Interaction between $Ras^{V12}$ and *scribbled* clones induces tumour growth and invasion. *Nature* **463**, 545–548 (2010).
52. Ohsawa, S. *et al.* Mitochondrial defect drives non-autonomous tumour progression through Hippo signalling in *Drosophila*. *Nature* **490**, 547–551 (2012).
53. Calbo, J. *et al.* A functional role for tumor cell heterogeneity in a mouse model of small cell lung cancer. *Cancer Cell* **19**, 244–256 (2011).
**This paper illustrated synergistic interactions between two populations of cancer cells that resulted in a novel metastatic phenotype of one of the populations.**
54. Zhang, M. *et al.* Intratumoral heterogeneity in a *Trp53*-null mouse model of human breast cancer. *Cancer Discov.* **5**, 520–533 (2015).
55. Kern, S. E. & Shibata, D. The fuzzy math of solid tumor stem cells: a perspective. *Cancer Res.* **67**, 8985–8988 (2007).
56. Duong, T. *et al.* VEGFD regulates blood vascular development by modulating SOX18 activity. *Blood* **123**, 1102–1112 (2014).
57. Inda, M. M. *et al.* Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. *Genes Dev.* **24**, 1731–1745 (2010).
58. Eirew, P. *et al.* Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. *Nature* **518**, 422–426 (2015).
**This paper demonstrated that intratumour subclonal heterogeneity is often maintained even after xenotransplantation thereby implying its evolutionary advantage.**
59. Kreso, A. *et al.* Variable clonal repopulation dynamics influence chemotherapy response in colorectal cancer. *Science* **339**, 543–548 (2013).
60. Maley, C. C. *et al.* Genetic clonal diversity predicts progression to esophageal adenocarcinoma. *Nat. Genet.* **38**, 468–473 (2006).
61. Notta, F. *et al.* Evolution of human *BCR–ABL1* lymphoblastic leukaemia-initiating cells. *Nature* **469**, 362–367 (2011).
62. Almendro, V. *et al.* Inference of tumor evolution during chemotherapy by computational modeling and *in situ* analysis of genetic and phenotypic cellular diversity. *Cell Rep.* **6**, 514–527 (2014).
63. Aceto, N. *et al.* Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. *Cell* **158**, 1110–1122 (2014).
64. Grant, P. R., Grant, B. R., Markert, J. A., Keller, L. F. & Petren, K. Convergent evolution of Darwin's finches caused by introgressive hybridization and selection. *Evolution* **58**, 1588–1599 (2004).
65. Snuderl, M. *et al.* Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. *Cancer Cell* **20**, 810–817 (2011).
66. Francis, J. M. *et al.* EGFR variant heterogeneity in glioblastoma resolved through single-nucleus sequencing. *Cancer Discov.* **4**, 956–971 (2014).
67. Anderson, K. *et al.* Genetic variegation of clonal architecture and propagating cells in leukaemia. *Nature* **469**, 356–361 (2011).
68. Greaves, M. & Maley, C. C. Clonal evolution in cancer. *Nature* **481**, 306–313 (2012).
69. Oesterreich, S. & Davidson, N. E. The search for *ESR1* mutations in breast cancer. *Nat. Genet.* **45**, 1415–1416 (2013).
70. Jeselsohn, R. *et al.* Emergence of constitutively active estrogen receptor-α mutations in pretreated advanced estrogen receptor-positive breast cancer. *Clin. Cancer Res.* **20**, 1757–1767 (2014).
71. Li, S. *et al.* Endocrine-therapy-resistant *ESR1* variants revealed by genomic characterization of breast-cancer-derived xenografts. *Cell Rep.* **4**, 1116–1130 (2013).
72. Toy, W. *et al. ESR1* ligand-binding domain mutations in hormone-resistant breast cancer. *Nat. Genet.* **45**, 1439–1445 (2013).
73. Robinson, D. R. *et al.* Activating *ESR1* mutations in hormone-resistant metastatic breast cancer. *Nat. Genet.* **45**, 1446–1451 (2013).
74. Vogelstein, B. & Kinzler, K. W. Digital PCR. *Proc. Natl Acad. Sci. USA* **96**, 9236–9241 (1999).
75. Hobor, S. *et al.* TGFα and amphiregulin paracrine network promotes resistance to EGFR blockade in colorectal cancer cells. *Clin. Cancer Res.* **20**, 6429–6438 (2014).
76. Thliveris, A. T. *et al.* Transformation of epithelial cells through recruitment leads to polyclonal intestinal tumors. *Proc. Natl Acad. Sci. USA* **110**, 11523–11528 (2013).
77. Fomchenko, E. I. *et al.* Recruited cells can become transformed and overtake PDGF-induced murine gliomas *in vivo* during tumor progression. *PLoS ONE* **6**, e20605 (2011).

78. Singh, R., Pochampally, R., Watabe, K., Lu, Z. & Mo, Y. Y. Exosome-mediated transfer of miR-10b promotes cell invasion in breast cancer. *Mol. Cancer* **13**, 256 (2014).
79. Gefen, O. & Balaban, N. Q. The importance of being persistent: heterogeneity of bacterial populations under antibiotic stress. *FEMS Microbiol. Rev.* **33**, 704–717 (2009).
80. Sharma, S. V. *et al.* A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* **141**, 69–80 (2010).
81. Oliveira, N. M., Niehus, R. & Foster, K. R. Evolutionary limits to cooperation in microbial communities. *Proc. Natl Acad. Sci. USA* **111**, 17941–17946 (2014).
82. Pepper, J. W., Scott Findlay, C., Kassen, R., Spencer, S. L. & Maley, C. C. Cancer research meets evolutionary biology. *Evol. Appl.* **2**, 62–70 (2009).
83. Pepper, J. W. Defeating pathogen drug resistance: guidance from evolutionary theory. *Evolution* **62**, 3185–3191 (2008).
84. Pienta, K. J., McGregor, N., Axelrod, R. & Axelrod, D. E. Ecological therapy for cancer: defining tumors using an ecosystem paradigm suggests new opportunities for novel cancer treatments. *Transl Oncol.* **1**, 158–164 (2008).
85. Pepper, J. W. Drugs that target pathogen public goods are robust against evolved drug resistance. *Evol. Appl.* **5**, 757–761 (2012).
**This paper used modelling to show how the elimination of pathogens can be achieved by removing common resources, thus indicating that abolishing of common gooder populations in cancers might lead to prolonged survival.**
86. Mateo, J., Gerlinger, M., Rodrigues, D. N. & de Bono, J. S. The promise of circulating tumor cell analysis in cancer management. *Genome Biol.* **15**, 448 (2014).
87. De Mattos-Arruda, L. *et al.* Circulating tumour cells and cell-free DNA as tools for managing breast cancer. *Nat. Rev. Clin. Oncol.* **10**, 377–389 (2013).
88. Haber, D. A. & Velculescu, V. E. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. *Cancer Discov.* **4**, 650–661 (2014).
89. Brown, R., Curry, E., Magnani, L., Wilhelm-Benartzi, C. S. & Borley, J. Poised epigenetic states and acquired drug resistance in cancer. *Nat. Rev. Cancer* **14**, 747–753 (2014).
90. Al-Dimassi, S., Abou-Antoun, T. & El-Sibai, M. Cancer cell resistance mechanisms: a mini review. *Clin. Transl Oncol.* **16**, 511–516 (2014).

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**Competing interests statement**
The authors declare competing interests: see Web version for details.