Published in final edited form as:
*Cancer Res*. 2012 October 1; 72(19): 4875–4882. doi:10.1158/0008-5472.CAN-12-2217.

# Intratumour Heterogeneity: Evolution through Space and Time

**Charles Swanton**[1,2]

[1]Cancer Research UK London Research Institute, Translational Cancer Therapeutics Laboratory, 44 Lincoln's Inn Fields, London WC2A 3LY

[2]UCL Cancer Institute, Paul O'Gorman Building, Huntley St., London WC1E 6BT

## Abstract

Recent technological advances have permitted higher resolution and more rapid analysis of individual cancer genomes at the single nucleotide level. Such advances have demonstrated bewildering inter-tumour heterogeneity with limited somatic alterations shared between tumours of the same histopathological subtype. Exacerbating such complexity, increasing evidence of intratumour genetic heterogeneity (ITH) is emerging, both within individual tumour biopsies and spatially separated between biopsies of the same tumour. Sequential analysis of tumours has also revealed evidence that ITH temporally evolves during the disease course. ITH has implications for predictive or prognostic biomarker strategies, where the tumour subclone that may ultimately influence therapeutic outcome may evade detection due to its absence or presence at low frequency at diagnosis or due to its regional separation from the tumour biopsy site. In this review the implications of "trunk and branch" tumour evolution for drug discovery approaches and emerging evidence that low frequency somatic events may drive tumour growth through paracrine signalling fostering a tumour ecological niche, are discussed. The concept of an "actionable mutation" is considered within a model of clonal dominance and heterogeneous tumour cell dependencies. Evidence that cancer therapeutics may augment ITH and the need to track the tumour subclonal architecture through treatment are defined as key research areas. Finally, if combination therapeutic approaches to limit the consequences of ITH prove challenging, identification of drivers or suppressors of ITH may provide attractive therapeutic targets to limit tumour evolutionary rates and adaptation.

## ITH: A new vision for an old problem

Tumour morphological heterogeneity has long been recognised by pathologists and forms the basis of many tumour grading prognostic classification systems. For example, one component of the breast cancer Scarff-Bloom-Richardson grading system is comprised of an assessment of nuclear pleomorphism, a characteristic associated with tumour aneuploidy (1). Indeed the term anaplasia, first coined by David Von Hansemann in 1890, refers to nuclear and mitotic atypia and reflects observations of tumour morphological heterogeneity. Tumour morphological heterogeneity is often regionally distinct with diversity in tumour cell proliferation, immune infiltration, differentiation status and necrosis that differ between microscopy fields.

As a result of these common pathological observations, ITH is considered in tumour immunohistochemical quantitative analyses that often take into account both the intensity of cellular staining as well as the percentage of tumour cells scoring positive for the immunohistochemical marker. Despite common histopathological observations of ITH in the clinical setting, our knowledge of the extent of ITH at the genetic or epigenetic level, as

Charles.swanton@cancer.org.uk.


Europe PMC Funders Author Manuscripts

well as its underlying causes, has remained relatively rudimentary. Conceptually, the prevalence of ITH and its underlying causes have not been advanced by the routine use of cancer genomics platforms due to the inability of expression microarray or DNA copy number platforms to resolve differences in mRNA expression or DNA copy number between cells of the same tumour.

The advent of tumour deep sequencing technologies, discussed in this review, has begun to resolve the extent of ITH at the single nucleotide level. Deep sequencing technologies are beginning to shed light on the consequences of such heterogeneity for drug discovery and biomarker validation approaches.

## An Evolutionary Perspective on Cancer Heterogeneity

The impact of heterogeneity on tumour growth control from an evolutionary perspective has been considered in detail (reviewed in (2)). In 1976 Peter Nowell proposed the clonal evolution model of cancer and applied evolutionary models to understand tumour growth and treatment failure and the phenomenon of increased tumour aggressiveness that occurs during the natural history of advanced solid tumours (3). Nowell noted a decade later in a reflection on his work “Tumors arise from a single “mutated” cell and that biological and clinical progression results from subsequent additional alterations, giving rise to more aggressive subpopulations within the original neoplastic clone”. He also noted that genetic instability, occurring in tumour cells during disease progression, might enhance this process.

Indeed, subsequent work in the early 80s from Harris, Chambers and Hill, investigating the generation of metastatic subclones from a mouse sarcoma line, concluded that the generation of such metastatic clones arose at a higher rate than the generation of stable mutations conferring drug resistance (10-1000 fold higher rate) and that acquisition of metastatic potential by subclones was in some cases reversible (4), coining the term “dynamic heterogeneity”. The parallels with Nowell’s concepts of genetic instability and disease progression are intriguing; Harris and colleagues concluded that metastatic variants generated from heterogeneous cell populations might arise through unknown epigenetic mechanisms or other mechanisms generating diversity at a higher rate. Subsequently the same group found that a highly metastatic melanoma cell line, B16F10 acquired resistance to methotrexate at a higher rate than the B16F1 line with low metastatic potential suggesting a common mechanism responsible for metastatic outgrowth and drug resistance, two common phenomena that co-occur in epithelial malignancies(5). The authors concluded that these phenotypes may be unified through one mechanism, mediated by the generation of heterogeneous structural chromosomal gene amplification events from cell to cell that are selected for during drug exposure or metastatic outgrowth.

Developments in the fields of mathematics and evolutionary biology are beginning to shed light on the impact of tumour diversity on evolutionary selection, raising important questions as to how advanced tumours might be more optimally controlled. Gatenby’s parallels of tumour metastatic growth with population growth control are prescient, “two centuries of experience have shown that the vast majority of introduced species are simply too heterogeneous, too dispersed and too adaptive to be eliminated”. He proposes an “adaptive therapy” approach, where drug sensitive clones are controlled, but not eliminated, to out-compete drug resistant but less fit subclones, may prove more tractable in the near-term to limit progression of advanced metastatic solid tumours (6, 7).

## Intratumor Heterogeneity in Space and Time

In contrast to linear models of tumour evolution with sequentially ordered somatic mutations in driver genes resulting in clonal sweeps of homogeneous tumour cell expansion, more

recent evidence suggests that branched evolutionary tumour growth may contribute to ITH both within a primary tumour and between primary and metastatic tumour sites (Figure 1).

Shah and colleagues investigated a case of advanced invasive lobular carcinoma of the breast and through whole genome sequencing demonstrated the existence of 19 non-synonymous mutations present in the metastasis that were not present in the primary tumour diagnosed 9 years before, illustrating the spatial and temporal dynamics of ITH (8). Through elegant FISH-based analyses of acute lymphoblastic leukemia single cells, Anderson and colleagues defined branched evolutionary growth and convergent evolution of recurrent copy number events occurring in different subclones of the same patient (9). In an extensive analysis of ITH in pancreatic cancer, Yachida and colleagues demonstrated that clonal tumour populations present in the primary, but genetically distinct from the non-metastatic clones, give rise to metastatic disease in a branched evolutionary pattern, with “progressor mutations” common to metastatic sites and within regionally separated subclones of the primary lesion (10). Campbell and colleagues demonstrated that genome instability occurs early in pancreatic cancer and contributes to on-going tumour evolution at metastatic sites, distinct from the primary, that may in turn seed tertiary metastases with evidence of convergent evolutionary paths and organ-specific relationships between metastases (11).

Navin et al have provided elegant insight into the depth and challenges of ITH in breast cancer within single tumour biopsies. Using a technique to separate tumour cells based on their DNA content, termed sector ploidy profiling, followed by DNA copy number analysis by CGH or single cell sequencing, they demonstrated that a single breast cancer biopsy may contain multiple intermixed karyotypic tumour populations that differ by major structural chromosomal gene amplifications. Such diversity may occur within one tumour biopsy or may be regionally separated, related through branched evolutionary growth (12, 13).

Our group analysed multiple regions of two primary clear cell renal carcinomas and associated metastatic sites (14). 63-69% of all non-synonymous somatic mutations identified across multiple biopsies of two primary tumours and their associated metastatic sites were not detectable in a single biopsy, suggesting that a single biopsy may underestimate the somatic mutational landscape of a tumour. We found evidence for ITH that was present at genetic, transcriptomic and functional levels with spatial separation of tumour subclones. Furthermore, between 25-50% of non-synonymous variants identified in 19 single biopsies across two tumours were private mutations and evaded detection elsewhere in the tumour despite sequencing to >250 fold coverage. Branched evolutionary growth was detected in this analysis with evidence of convergent evolution, with multiple recurrent, yet distinct, inactivating mutations occurring in the same tumour suppressor genes including SETD2, PTEN and KDM5C in different branches (and regions) of the tumour phylogenetic tree. Therefore, evidence now suggests that despite considerable ITH, it appears there are recurrent targets that are subject to loss of function mutations and convergent evolution, suggesting deterministic tumour dependencies that may be exploitable (9, 14).

Substantiating conclusions of branched tumour growth, Stratton and Campbell and colleagues have provided an in-depth whole genome sequencing analysis of 21 breast cancers (15, 16). The authors confirm subclonal variation and demonstrate that the majority of tumour somatic mutations occur following tumour diversification and branching. Strikingly, all tumours harbored a dominant clone (>50% of cancer cells) that differed by thousands of mutations from other subclones. For example, one tumour harboured a dominant clone with 15,600 mutations distinct from subclones within other branches of the phylogenetic tree. By inference the authors conclude that the proliferation and eventual outgrowth of the subclone, precipitating mammographic detection, must have been a rate

limiting event due to the vast number of mutations present in that subclone that differ from the common somatic events present in all tumour cells.

## Intratumour Heterogeneity and Evolutionary Bottlenecking

Through the analysis of structural variations and allelic frequencies in a primary basal breast cancer, a xenograft and a brain metastasis from the same patient, Ding et al demonstrated that the metastasis may derive from a low frequency subclone within the primary (17). The team noted a wide range of allelic variant frequencies in the primary tumour, indicative of substantial ITH, with less divergent mutational frequencies at the metastatic site, suggesting a process analogous to evolutionary bottlenecking through subclonal selection during the metastatic process (18). Substantiating the capacity for such heterogeneity to foster metastatic growth, medulloblastoma metastases from the same patient are relatively homogeneous and derive from a low frequency subclone of the primary tumour (19).

Through our multiregion sequencing analysis of ccRCCs, metastases could be traced back to a distinct region of the primary tumour (14). Consistent with observations from Ding and colleagues, there appeared to be a relative restriction of diversity at metastatic sites (17). Sector ploidy and allelic imbalance analysis of the tumour and metastatic sites suggested that the primary region that spawned the metastasis had become tetraploid with the metastatic sites harbouring a chromosomally unstable pattern by allelic imbalance analysis with structural chromosomal complexities that differed between regions of the same metastatic site (Figure 2). Conceivably, following subclonal selection and the restriction of diversity and bottlenecking, generation of tumour chromosomal instability (CIN) provides a route to rapidly initiate a further expansion in tumour heterogeneity. It is tempting to speculate that this may explain observations of increased CIN at metastatic sites to compensate for the transient restriction of diversity during the selection of a minority tumour subclone (20). The parallels with Harris' work on dynamic heterogeneity are notable since genomic instability driven by heterogeneous structural and numerical chromosomal changes, promoting extensive alterations in gene dosage, may be one such way of creating non-mutational routes to tumour metastases (4, 5). Conceivably, such mechanisms may also be reversible, as postulated in the dynamic heterogeneity model, since distinct alterations in gene dosage permissive for metastatic outgrowth may be lost in subsequent cell divisions due to spontaneous chromosome re-assortments.

## The Trunk-Branch Model of Tumour Growth: Delineating heterogeneous from ubiquitous events

Modelling tumour diversity within a tree structure of tumour growth provides a conceptual framework to consider the capacity of tumours to evade cancer therapeutics and the limitations to current biomarker validation strategies (Figure 1). Early somatic events that drive tumour growth or maintenance, present in early clonal progenitors are represented within the "trunk" of the tumour (21). For example, somatic events in VHL (ccRCC) or p53 (triple negative breast cancer) are often clonally dominant, mapping to the trunk of the tumour evolutionary tree (14, 22). Such trunk somatic aberrations, present at the early stages of tumour development are likely to be ubiquitous events occurring at all sites of disease. In contrast, later somatic events that occur following branched separation of subclones represent heterogeneous events. Such subclonal heterogeneity may occur within a single biopsy or may be spatially separated between regions of the same tumour or its metastatic sites (14, 21). Intriguingly, evidence is emerging that indicates commonly accepted early drivers of disease biology such as VHL loss (23) and p53, PTEN or PIK3CA somatic mutations in triple negative breast cancer (22) may not always be clonally dominant in the




Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

tumour population or present in all the tumour cells (p53, PTEN and PIK3CA in TNBC) or primary and metastatic regions sampled (VHL in renal cancer).

## Clinical Implications of ITH

### A. Tumour Sampling Bias

Tumour sampling bias may arise due to differences in somatic events within the primary tumour, between the primary and metastatic sites, between metastatic sites or even within single biopsies (Figure 1). Heterogeneity is also dynamic and evolves over time, as has been observed in elegant FISH studies in cytogenetically high-risk multiple myeloma (24). Dynamic changes in the subclonal architecture of the tumour, where tumour subclones may change and compete with each other for dominance during the disease course and through treatment, creates challenges for predictive or prognostic biomarker efforts where the tumour subclone that defines clinical outcome (eg secondary plasma cell leukemia) may not be readily detectable at diagnosis (24).

Therapeutic decision-making in oncological practice is often made with reference to the primary tumour lesion, diagnosed months or years previously, or in cases where patients present with advanced disease, from one metastatic site. Such approaches are likely to be therapeutically tractable if these tumour somatic events occur in the tumour trunk and are present ubiquitously throughout all tumour subclones and continue to maintain tumour growth and survival at all sites of disease. However, the changing dynamics of tumour subclonal architecture over space and time, may result in previously sub-dominant clones, perhaps either not present or barely detectable in the primary, gaining pre-eminence. Conceivably, differences in tumour environmental selection pressures at metastatic sites may result in regional variation of tumour subclone evolution as distinct environments select for certain subclones over others, further contributing to ITH (11). Therefore, alterations in the subclonal architecture of the tumour may result in changes in tumour molecular profile that may differ between sites of metastatic disease, distinct from the profile of the primary tumour.

Qualification of clinical biomarkers has been a notoriously difficult process with only 100 of the estimated 150,000 biomarkers qualified and implemented into clinical practice (25). Biomarker discovery approaches combining laboratory analyses of gene function with genetic or transcriptomic analyses of tumour tissue often rely on single tumour biopsies of the primary or metastatic lesions to prioritise the identification of candidate biomarkers for validation (26). Conceivably, a highly variable genetic and transcriptomic landscape across a primary and metastatic tumour, might lead to tumour sampling bias confounding the validation of biomarkers due to spurious associations of heterogeneous tumour genetic events with clinical outcome in the discovery phase of these studies.

### B. Capacity for therapy to augment ITH

In contrast to mutator phenotypes and microenvironmental factors such as tissue hypoxia and acidosis that may enhance ITH (27), the capacity for cytotoxic therapies to augment ITH remains relatively underexplored. In the study by Keats and colleagues, the enhanced complexity of the relapsed subclone suggested that treatment with DNA damaging agents through myeloma therapy may potentially exacerbate genomic complexity (24). The potential for therapy to augment genome instability and ITH has been observed in relapsed MGMT-deficient glioblastoma multiforme following alkylator therapy that provides a selection pressure to lose DNA mismatch repair function (28). Similarly, cytotoxic therapy was thought to be responsible for the increase in transversions witnessed in relapsed AML following exposure to DNA damaging agents (29). Given the association of ITH with poorer

clinical outcome (30), such observations suggest the controversial concept that therapeutics may in some cases contribute to enhanced tumour diversity and adaptation.

Therefore, cancer cytotoxics may contribute to an enhanced tumour evolutionary rate through fostering genetic diversity and as proposed by Gillies et al, by initiating an adverse tumour microenvironment that enables small phenotypic changes to result in large variations in fitness (27).

### C. Defining actionable mutations within a model of clonal dominance

Tumour deep sequencing analyses have led to attempts to stratify therapeutics based on the occurrence of “actionable mutations” where a clinician matches a tumour mutation to a cancer drug. Somatic mutational heterogeneity, where distinct mutations may be present in some biopsies but not detectable in others, suggests a more cautionary approach to the clinical implementation of deep sequencing in circumstances where previously undescribed and unvalidated “actionable mutations” might be considered as suitable for clinical trial stratification. Given observations of the spatial separation of somatic mutations, it is conceivable that some actionable mutations, particularly ones with limited prior evidence of relevance in a disease subtype, may not be present within all tumour cells (present at submodal frequencies) or may be regionally separated, and thus represent relatively poor therapeutic targets (Figure 1). Similarly, synthetic lethal approaches to drug discovery are likely to have optimal efficacy if the genetic dependency of the drug discovery approach occurs early in tumour evolution, present in all tumour subclones, as witnessed by the potent efficacy of PARP inhibition in tumours of BRCA germline carriers(31).

Perhaps, within the context of early phase clinical trial development, consideration could be given to the clonal dominance of such “actionable mutations”, since sampling multiple sites of disease, to establish the ubiquitous nature of such mutations, is not only traumatic for the patient but also impractical clinically. For example, recent evidence, in glioblastoma and triple negative breast cancer, indicates that commonly accepted “actionable mutations” in signal transduction pathway regulators such as PTEN or PIK3CA may represent somatic events occurring in the branches of the tumour rather than clonally dominant ubiquitous, trunk mutations (22, 32).

Such considerations should not imply that genetic approaches to treatment stratification from single biopsies are futile. Many of the established biomarkers in oncology derive from, and have been clinically qualified in, single tumour biopsies. Examples include HER2 amplification or over-expression to guide trastuzumab therapy in breast cancer, BRAFV600E mutation to guide BRAF inhibitor treatment in melanoma and EGFR activating mutations to guide gefitinib or erlotinib treatment in adenocarcinoma of the lung.

It is likely that many established drivers of disease biology occur early in tumour development and represent ubiquitous events in the “trunk” of the tumour, present at all sites of disease, less subject to tumour sampling bias (21). In the case of renal cancer, such trunk events would be represented by VHL or PBRM1 somatic mutations (14). However, low frequency somatic events present in some subclones but not others (analogous to the branches of the tumour tree), generating ITH, are likely to contribute to the acquisition of drug resistance driven by Darwinian selection through treatment. Low frequency gatekeeper mutations in the EGFR tyrosine kinase are associated with inferior progression free survival on EGFR tyrosine kinase inhibitor therapy(33). Similarly, NSCLC exposure to EGFR TKIs can result in the selection of resistant subclones harbouring low frequency MET amplification, present in the tumour before treatment (34).




Europe PMC Funders Author Manuscripts

Such examples suggest that biomarker efforts may have to adapt to the challenge of detecting heterogeneous somatic events present in the tumour at low frequency in order to predict therapeutic outcome or define combination approaches to limit the acquisition of drug resistance as well as understand the complex phenotypic interplay between heterogeneous cancer subclones. ITH supports a more cautious approach when defining the presence of an actionable mutation for clinical trial stratification to encompass a definition that incorporates clonal or inter-regional tumour dominance.

### D. Cancer as a Complex Ecological System Dependent on Population Level Heterogeneity

The clonal dominance model to define an actionable event, does not necessarily preclude the possibility that tumour growth can be limited through the targeting of heterogeneous, low frequency subpopulations. Evidence suggests that minority cancer cell populations may maintain ITH and by inference, targeting such low frequency cells might impact upon the bulk tumour population; low frequency glioblastoma subclones harbouring mutated EGFR maintain ITH through paracrine activation of proliferation of the EGFR-wild type cancer cell population (35). Considering tumours as ecological niches with complex functional interdependencies may be necessary to further refine definitions of actionable mutations to attenuate tumour growth. Taking advantage of ITH through enforced competition between tumour subclones forms the basis of elegant models of tumour adaptive therapy (7).

### E. Cancer Cell Phenotypic Heterogeneity and Drug Sensitivity

ITH is likely to have direct phenotypic consequences on tumour behaviour and the acquisition of drug resistance. For example, low frequency subclones, detectable in the tumour prior to treatment, harbouring resistance mutations in EGFR in NSCLC (33) as well as multiple distinct secondary mutations in c-KIT in different metastases that occur in GIST following KIT/PDGFRA tyrosine kinase inhibitor therapy (36) confer resistance to targeted approaches.

Our group has shown that a heterogeneous mutation near the kinase domain of mTOR promotes resistance to serum deprivation and hyper-activation of the mTOR pathway following Everolimus therapy in regions of the primary tumour that harbour the mutation, but not in primary tumour regions with wild-type mTOR. Similarly, extensive ITH in DNA copy number events of “drivers” such as MET, PDGFRA and EGFR have been shown to occur in a mutually exclusive manner in glioblastoma(37). Adjacent GBM tumour cells display distinct copy number abnormalities in these “targetable” or actionable receptor tyrosine kinase amplification events. Heterogeneous copy number events present in the tumour branches, rather than trunk, suggest that targeting individual branched genetic lesions may prove relatively futile if ITH results in phenotypic heterogeneity in drug sensitivity. Szerlip and colleagues have confirmed this by demonstrating that cell lines grown from the same glioblastoma with heterogeneous PDGFR or EGFR amplification states require both PDGFR and EGFR inhibition for maximal PI3K pathway attenuation and growth inhibition (38). Intriguingly such heterogeneity is likely to be maintained and selected for by the presence of double-minute chromosomes harbouring RTK amplification. Such double-minute chromosomes lack centromeres and are therefore unequally segregated during mitosis resulting in the propagation of ITH. Phenotypic heterogeneity in drug sensitivity profiles that may be spatially separated or present at low frequency in minor subclones of the tumour, suggests that profiling cancer cell phenotypes from single biopsies to guide therapeutic decision-making from heterogeneous tumours may prove challenging.

In summary, whilst a proportion of tumours of the same histopathological subtype may share common drivers, branched events initiating heterogeneity in potential resistance pathways to targeted therapeutics will likely result in the need to consider individual

tumour-specific strategies to extend progression-free intervals. A future where personalised-medicine moves from the current status of patient cohorts defined by single “trunk” tumour driver events, to the single patient where both trunk and branch tumour events are characterised in advance of treatment, where no two tumours share the same characteristics, may be envisaged. This may have important regulatory, ethical and health economic implications and raises the need to incorporate an understanding of tumour heterogeneity into clinical trial design. For these reasons, some rightly argue that focussing on the consequences of genetic diversity in terms of common tumour phenotypes, rather than the specific genetic aberrations themselves, may prove more beneficial (27).

### F. Relationships between ITH and Clinical and Pathological Parameters

The presence of extensive ITH within primary RCCs suggests a rational basis for the improvements in survival outcome associated with palliative surgery to the primary site in patients with oligometastatic disease, through the removal of an evolutionary sink of primary tumour diversity with the capacity to seed further metastases. Longitudinal studies are primed to reveal further insight regarding the extent to which metastatic sites represent outgrowth of multiple heterogeneous subclones from the primary tumour and genetic events that may be permissive for metastatic outgrowth during the bottlenecking process.

Despite the emerging consequences of ITH on tumour adaptation, little is known about the relationship of ITH with standard tumour histopathological prognostic parameters, nor how mechanisms generating ITH vary between primary tumours and metastatic recurrences. Developing robust methods to define ITH will be a critical step in this process (Figure 1). Extensive evidence supports the association of clonal diversity with progression from pre-invasive to invasive adenocarcinoma and chromosomal instability with poorer disease outcome (30, 39, 40). Prospective analyses of the association of ITH in the primary tumour with risk of early metastatic relapse following adjuvant cytotoxic or radiotherapy seem justified. Such approaches will address whether the degree of ITH might shed light on our ability to cure some primary tumours but not others.

## Future Directions

ITH provides a necessary substrate for Darwinian selection during metastatic outgrowth and therapeutic resistance. Subclonal selection and transient bottlenecking that has been shown to occur during these processes provides both a tool to decipher potential permissive genetic events required during this process and a therapeutic opportunity, if these steps are governed by a restricted set of actionable mutations. Developing minimally invasive approaches to track and monitor tumour subclonal dynamics through the disease course will be an essential step in this process that will also allow the extent to which cytotoxic therapies may exacerbate genomic instability and ITH to be monitored.

Multi-region and ultra-deep sequencing analyses have the potential to shed light on further convergent evolutionary events that tumours must overcome in order to maintain or continue growth, as evidenced by recurrent distinct SETD2 mutations in ccRCC. Such “predictable” and deterministic tumour dependencies may represent new therapeutic opportunities to mitigate the risk of ITH. Drawing parallels in cancer with examples of convergent evolution in ecology, emphasises the continued need to consider tumour growth within evolutionary and population genetics models (41). Such convergent evolutionary events highlight the requirement to prioritise research on the ecological tumour niche as the selection force (and in some cases the driver itself of genomic instability) for genetic adaptation, as proposed by Gatenby (41).

Europe PMC Funders Author Manuscripts

Continued distinction between trunk and branch events may expand the repertoire of actionable mutations in the tumour trunk, albeit in smaller and smaller patient cohorts. By illuminating common branched events that may predispose to therapeutic failure through subclonal outgrowth, novel combinatorial therapeutic strategies may be envisaged to short-circuit future tumour evolutionary networks and drug resistance mechanisms. ITH may present profound regulatory and practical clinical challenges when considering such drug combinatorial approaches, faced with a restricted number of drugs active against defined actionable mutations compared to the bewildering potential for diversity within individual tumours.

Finally, if such approaches prove intractable, hope may derive from functional studies that illuminate new classes of suppressors and initiators of tumour diversity and immunotherapeutic approaches targeting tumour neo-antigenic diversity, that may ultimately lead to therapeutic opportunities to limit tumour adaptation and Darwinian selection.

## References


1. Frierson HF Jr. Grade and flow cytometric analysis of ploidy for infiltrating ductal carcinomas. Hum Pathol. 1993; 24(1):24–9. Epub 1993/01/01. [PubMed: 8380276]
2. Greaves M, Maley CC. Clonal evolution in cancer. Nature. 2012; 481(7381):306–13. Epub 2012/01/20. [PubMed: 22258609]
3. Nowell PC. The clonal evolution of tumor cell populations. Science. 1976; 194(4260):23–8. Epub 1976/10/01. [PubMed: 959840]
4. Harris JF, Chambers AF, Hill RP, Ling V. Metastatic variants are generated spontaneously at a high rate in mouse KHT tumor. Proc Natl Acad Sci U S A. 1982; 79(18):5547–51. Epub 1982/09/01. [PubMed: 6957880]
5. Cillo C, Dick JE, Ling V, Hill RP. Generation of drug-resistant variants in metastatic B16 mouse melanoma cell lines. Cancer Res. 1987; 47(10):2604–8. Epub 1987/05/15. [PubMed: 3567893]
6. Gatenby RA. A change of strategy in the war on cancer. Nature. 2009; 459(7246):508–9. Epub 2009/05/30. [PubMed: 19478766]
7. Gatenby RA, Silva AS, Gillies RJ, Frieden BR. Adaptive therapy. Cancer Res. 2009; 69(11):4894–903. Epub 2009/06/03. [PubMed: 19487300]
8. Shah SP, Morin RD, Khattra J, Prentice L, Pugh T, Burleigh A, et al. Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. Nature. 2009; 461(7265):809–13. Epub 2009/10/09. [PubMed: 19812674]
9. Anderson K, Lutz C, van Delft FW, Bateman CM, Guo Y, Colman SM, et al. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature. 2011; 469(7330):356–61. Epub 2010/12/17. [PubMed: 21160474]
10. Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature. 2010; 467(7319):1114–7. Epub 2010/10/29. [PubMed: 20981102]
11. Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, Stebbings LA, et al. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature. 2010; 467(7319): 1109–13. Epub 2010/10/29. [PubMed: 20981101]
12. Navin N, Krasnitz A, Rodgers L, Cook K, Meth J, Kendall J, et al. Inferring tumor progression from genomic heterogeneity. Genome Res. 2010; 20(1):68–80. Epub 2009/11/12. [PubMed: 19903760]
13. Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, et al. Tumour evolution inferred by single-cell sequencing. Nature. 2011; 472(7341):90–4. Epub 2011/03/15. [PubMed: 21399628]
14. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012; 366(10):883–92. Epub 2012/03/09. [PubMed: 22397650]
15. Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, et al. Mutational Processes Molding the Genomes of 21 Breast Cancers. Cell. 2012 Epub 2012/05/23.

16. Nik-Zainal S, Van Loo P, Wedge DC, Alexandrov LB, Greenman CD, Lau KW, et al. The Life History of 21 Breast Cancers. Cell. 2012 Epub 2012/05/23.
17. Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW, et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature. 2010; 464(7291):999–1005. Epub 2010/04/16. [PubMed: 20393555]
18. Gerlinger M, Swanton C. How Darwinian models inform therapeutic failure initiated by clonal heterogeneity in cancer medicine. Br J Cancer. 2010; 103(8):1139–43. Epub 2010/09/30. [PubMed: 20877357]
19. Wu X, Northcott PA, Dubuc A, Dupuy AJ, Shih DJ, Witt H, et al. Clonal selection drives genetic divergence of metastatic medulloblastoma. Nature. 2012; 482(7386):529–33. Epub 2012/02/22. [PubMed: 22343890]
20. Carter SL, Eklund AC, Kohane IS, Harris LN, Szallasi Z. A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. Nat Genet. 2006; 38(9):1043–8. [PubMed: 16921376]
21. Yap T, Gerlinger M, Futreal A, Pustzai L, Swanton C. Intratumour Heterogeneity: Seeing the wood for the trees. Science Translational Medicine. 2012 In press 2012.
22. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature. 2012 Epub 2012/04/13.
23. Vaziri SA, Tavares EJ, Golshayan AR, Rini BI, Aydin H, Zhou M, et al. Differing von Hippel Lindau genotype in paired primary and metastatic tumors in patients with clear cell renal cell carcinoma. Frontiers in Genitourinary Oncology. 2012; 2(51) doi: 10.3389.
24. Keats JJ, Chesi M, Egan JB, Garbitt VM, Palmer SE, Braggio E, et al. Clonal competition with alternating dominance in multiple myeloma. Blood. 2012 Epub 2012/04/14.
25. Poste G. Bring on the biomarkers. Nature. 2011; 469(7329):156–7. Epub 2011/01/14. [PubMed: 21228852]
26. Swanton C, Larkin JM, Gerlinger M, Eklund AC, Howell M, Stamp G, et al. Predictive biomarker discovery through the parallel integration of clinical trial and functional genomics datasets. Genome Med. 2010; 2(8):53. Epub 2010/08/13. [PubMed: 20701793]
27. Gillies RJ, Verduzco D, Gatenby RA. Evolutionary dynamics of carcinogenesis and why targeted therapy does not work. Nat Rev Cancer. 2012; 12(7):487–93. Epub 2012/06/15. [PubMed: 22695393]
28. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008; 455(7216):1061–8. Epub 2008/09/06. [PubMed: 18772890]
29. Ding L, Ley TJ, Larson DE, Miller CA, Koboldt DC, Welch JS, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature. 2012; 481(7382):506–10. Epub 2012/01/13. [PubMed: 22237025]
30. McGranahan N, Burrell RA, Endesfelder D, Novelli MR, Swanton C. Cancer chromosomal instability: therapeutic and diagnostic challenges. ‘Exploring aneuploidy: the significance of chromosomal imbalance’ review series. EMBO Rep. 2012 Epub 2012/05/19.
31. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009; 361(2):123–34. Epub 2009/06/26. [PubMed: 19553641]
32. Nickel GC, Barnholtz-Sloan J, Gould MP, McMahon S, Cohen A, Adams MD, et al. Characterizing mutational heterogeneity in a glioblastoma patient with double recurrence. PLoS ONE. 2012; 7(4):e35262. Epub 2012/04/27. [PubMed: 22536362]
33. Su KY, Chen HY, Li KC, Kuo ML, Yang JC, Chan WK, et al. Pretreatment Epidermal Growth Factor Receptor (EGFR) T790M Mutation Predicts Shorter EGFR Tyrosine Kinase Inhibitor Response Duration in Patients With Non-Small-Cell Lung Cancer. J Clin Oncol. 2012; 30(4):433–40. Epub 2012/01/05. [PubMed: 22215752]
34. Turke AB, Zejnullahu K, Wu YL, Song Y, Dias-Santagata D, Lifshits E, et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. Cancer Cell. 2010; 17(1):77–88. Epub 2010/02/05. [PubMed: 20129249]

35. Inda MM, Bonavia R, Mukasa A, Narita Y, Sah DW, Vandenberg S, et al. Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. Genes Dev. 2010; 24(16):1731–45. Epub 2010/08/18. [PubMed: 20713517]

36. Liegl B, Kepten I, Le C, Zhu M, Demetri GD, Heinrich MC, et al. Heterogeneity of kinase inhibitor resistance mechanisms in GIST. J Pathol. 2008; 216(1):64–74. Epub 2008/07/16. [PubMed: 18623623]

37. Snuderl M, Fazlollahi L, Le LP, Nitta M, Zhelyazkova BH, Davidson CJ, et al. Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. Cancer Cell. 2011; 20(6): 810–7. Epub 2011/12/06. [PubMed: 22137795]

38. Szerlip NJ, Pedraza A, Chakravarty D, Azim M, McGuire J, Fang Y, et al. Intratumoral heterogeneity of receptor tyrosine kinases EGFR and PDGFRA amplification in glioblastoma defines subpopulations with distinct growth factor response. Proc Natl Acad Sci U S A. 2012; 109(8):3041–6. Epub 2012/02/11. [PubMed: 22323597]

39. Maley CC, Galipeau PC, Finley JC, Wongsurawat VJ, Li X, Sanchez CA, et al. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. Nat Genet. 2006; 38(4):468–73. Epub 2006/03/28. [PubMed: 16565718]

40. Birkbak NJ, Eklund AC, Li Q, McClelland SE, Endesfelder D, Tan P, et al. Paradoxical relationship between chromosomal instability and survival outcome in cancer. Cancer research. 2011; 71(10):3447–52. Epub 2011/01/29. [PubMed: 21270108]

41. Gatenby RA, Gillies RJ, Brown JS. Of cancer and cave fish. Nat Rev Cancer. 2011; 11(4):237–8. Epub 2011/05/10. [PubMed: 21548400]

Europe PMC Funders Author Manuscripts

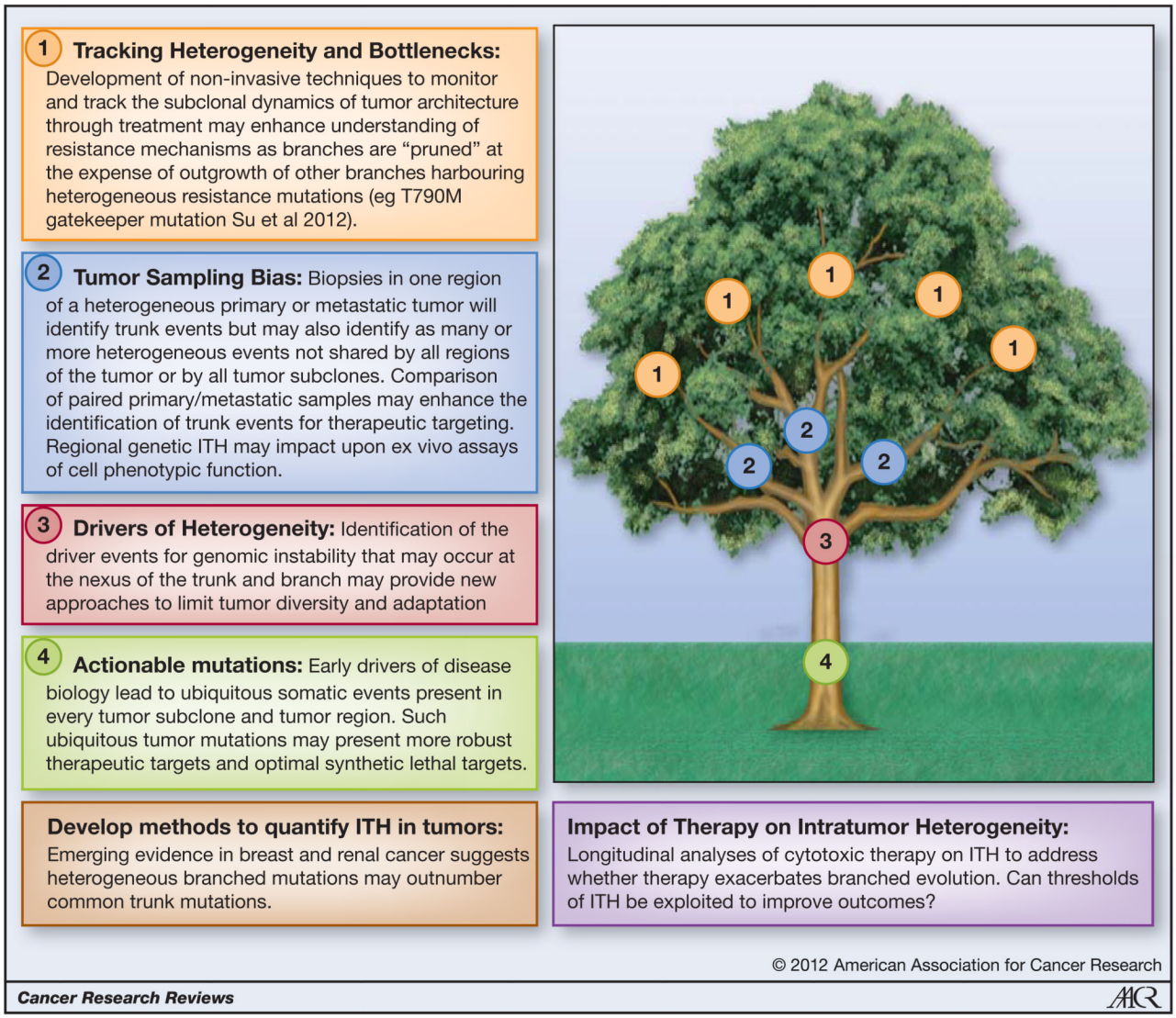


**Figure 1. Trunk and Branch model representing intratumour heterogeneity**

Common or ubiquitous events in the tumour found in every subclone and every tumour region are represented in the trunk of the tree. Diverse, heterogeneous somatic events are represented by the branches and the leaves. Tumour somatic events occurring in the trunk or branches may be driver or passenger events that may be dynamic during tumour evolution and adaptation to therapy (Adapted from Yap et al 2012). The figure illustrates key research areas and the need to identify how cancer therapeutics might influence intratumour heterogeneity.

Europe PMC Funders Author Manuscripts

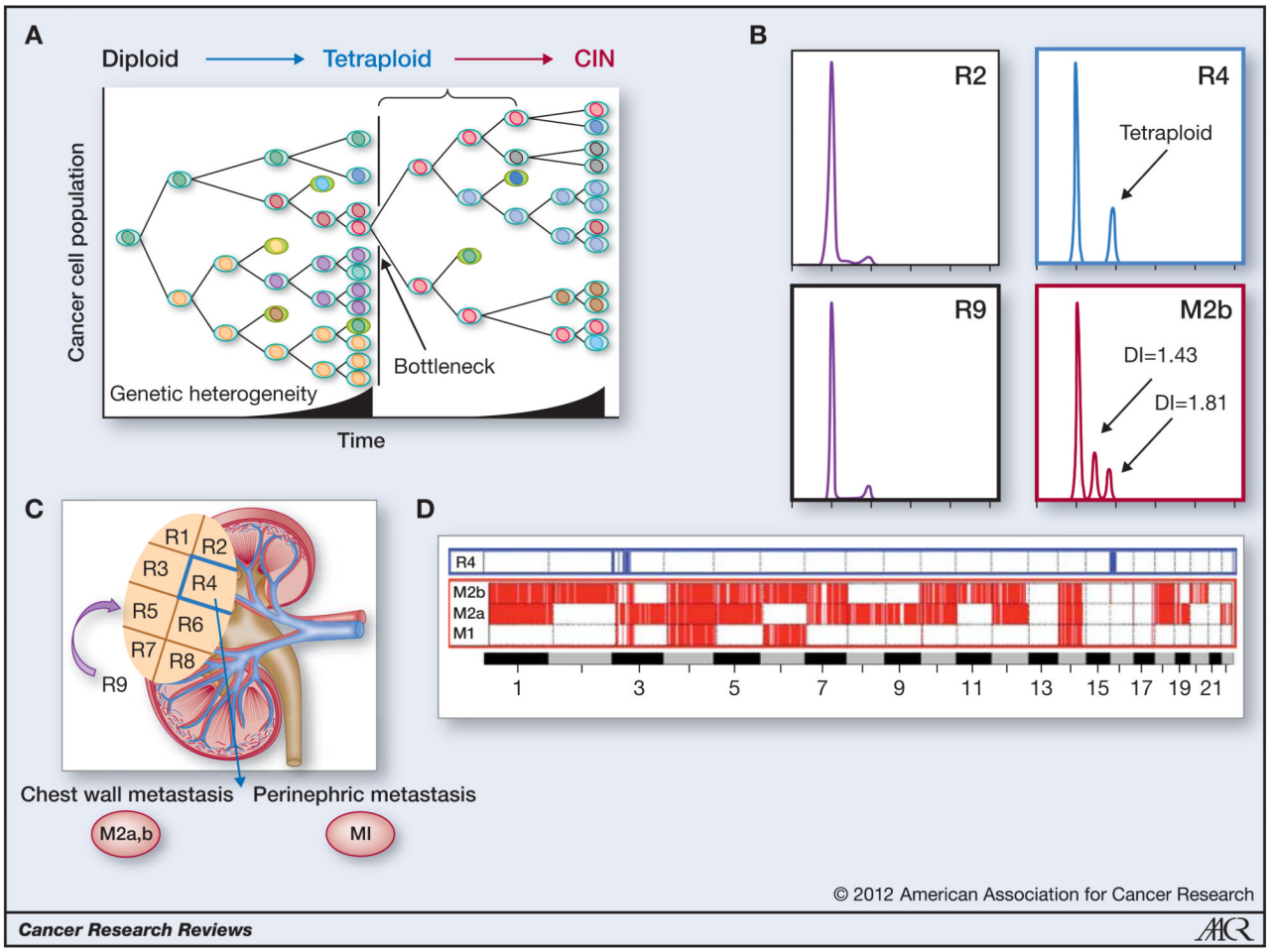


**Figure 2. Evolutionary Bottlenecking and Restriction of Diversity between primary and metastases**

**(A)** Transient restriction of diversity through an evolutionary bottlenecking process, eg during clonal selection through therapy or during metastatic progression may lead to the requirement for alternative mechanisms to generate ITH. One such mechanism can be generated through structural and whole chromosomal instability (CIN). Tetraploidy is thought to be a precursor of chromosomal instability. (**B/C**) Multi-region analysis of a primary renal cell carcinoma and its metastatic sites revealed the metastatic lesions were most similar to Region 4 of the primary (adapted from Gerlinger et al 2012). Ploidy analysis revealed that the primary regions were all diploid with the exception of region 4 which was tetraploid. Metastatic sites were sub-tetraploid. **(D)** Allelic imbalance analysis revealed substantial heterogeneity in chromosome structure between two biopsies of the same metastatic site (M2a and M2b). Taken together with ploidy analysis, these data indicate the possible emergence of CIN at metastatic sites. Longitudinal studies will reveal to what extent metastatic sites represent outgrowth of multiple heterogeneous subclones from the primary tumour and genetic events that may be permissive for metastatic outgrowth during the bottlenecking process. Figure 2 Adapted from references 14 and 18 (Gerlinger et al NEJM 2012 and Gerlinger et al 2010 British Journal of Cancer).