Biochimica et Biophysica Acta 1855 (2015) 264–275

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Inferring mutational timing and reconstructing tumour evolutionary histories

Samra Turajlic [a], Nicholas McGranahan [a], Charles Swanton [a,b,*]

[a] The Francis Crick Institute, 44 Lincoln's Inn Fields, London WC2A 3LY, UK
[b] UCL Cancer Institute, CRUK Lung Cancer Centre of Excellence, Huntley Street, WC1E 6DD, UK

ARTICLE INFO

Article history:
Received 2 January 2015
Received in revised form 17 March 2015
Accepted 19 March 2015
Available online 27 March 2015

Keywords:
Mutational timing
Intratumour heterogeneity
Cancer evolution
Subclonal mutations
Treatment resistance

ABSTRACT

Cancer evolution can be considered within a Darwinian framework. Both micro and macro-evolutionary theories can be applied to understand tumour progression and treatment failure. Owing to cancers' complexity and heterogeneity the rules of tumour evolution, such as the role of selection, remain incompletely understood. The timing of mutational events during tumour evolution presents diagnostic, prognostic and therapeutic opportunities. Here we review the current sampling and computational approaches for inferring mutational timing and the evidence from next generation sequencing-informed data on mutational timing across all tumour types. We discuss how this knowledge can be used to illuminate the genes and pathways that drive cancer initiation and relapse; and to support drug development and clinical trial design.

Contents

* Corresponding author.
E-mail address: Charles.swanton@crick.ac.uk (C. Swanton).

http://dx.doi.org/10.1016/j.bbcan.2015.03.005

1. Introduction

Genetic intratumour heterogeneity (ITH) has long been observed in a variety of cancers, but its extent at the level of the single cell and single nucleotide is only now becoming apparent with the use of next generation sequencing technologies (reviewed in [1,2]). Branched tumour evolution gives rise to multiple genetically distinct subclones which may co-exist in the tumour mass and produce varying degrees of ITH [3]. A recent study in colorectal cancer shows that most detectable ITH occurs early after the transition to an advanced tumour as a result of fairly neutral subclonal evolution [4]. Critically, when heterogeneous tumours are sampled *via* a single biopsy the true extent of ITH is underestimated [5]. Intra-tumour diversity has rightly been perceived as a significant problem, but on the plus side, by informing phylogenetic analyses it can help to reconstruct tumour histories and estimate mutational timing (Fig. 1).

Clonal mutations (also termed: ubiquitous, shared or founder mutations) are present in all the malignant cells under examination and are placed on the trunk of the phylogenetic trees (Fig. 1: truncal mutations). The most-recent common ancestor, which may no longer be detectable in the population which is sampled, is characterised by mutations that are fully clonal. Subclonal mutations are present in a proportion of the malignant cells under examination and map to the branches of phylogenetic trees (Fig. 1: branch mutations). Subclonal populations, or subclones, are characterised by the presence of at least one subclonal mutation. In a phylogenetic tree the distance between a subclone and the most-recent common ancestor indicates the degree of their relationship (Fig. 1) and provides an approximation of mutational timing: trunk mutations occur prior to branch mutations. In what regard is this information meaningful or useful? At the most basic level the knowledge of mutational timing can illuminate the critical steps in tumourigenesis and identify mutational events linked to tumour initiation, maintenance and progression and resolve the timing of specific mutational or genomic instability processes during tumour evolution.

Next, mutational timing can help distinguish driver and passenger mutations. Recent analyses suggest that half or more of the somatic mutations in tumours of self-renewing tissues occur before malignant transformation [6]. By identifying mutations in pre-malignant, or *in-situ* lesions, a significant number of candidate driver mutations can be eliminated. For example, in a genome-wide analysis of progression from Barrett's oesophagus to invasive oesophageal adenocarcinoma, the majority of mutations accumulated early, prior to tumour initiation, and only two mutations were linked to progression from the pre-malignant to malignant state [7]. However useful, these strategies are only applicable in tumour types where early lesions can be readily identified and sampled.

The knowledge of mutational timing has a potential clinical utility. In clear cell renal cell carcinoma (ccRCC) clonal events are very consistent (VHL, loss of 3p) [8] and yet the patient outcomes are diverse, suggesting that late/subclonal events influence the disease course. Consistent with this, the presence of subclones has been linked to poor clinical outcome in chronic lymphocytic leukaemia (CLL) [9], disease relapse in lung adenocarcinoma [10] and to increased risk of progression to malignancy in Barrett's oesophagus [11]. The order in which *JAK2* and *TET2* mutations are acquired can influence clinical features, response to therapy and clonal evolution in myeloproliferative neoplasms [12].

Although the within-tumour clonal dynamics can be effectively-neutral in some cancer types [4,13] selective bottlenecks will occur

Clone	Mutations					
	A	B	C	D	E	F
C1	■	■				
C2	■	■	■			■
C3	■	■	■			
C4	■	■		■		
C5	■	■	■	■	■	

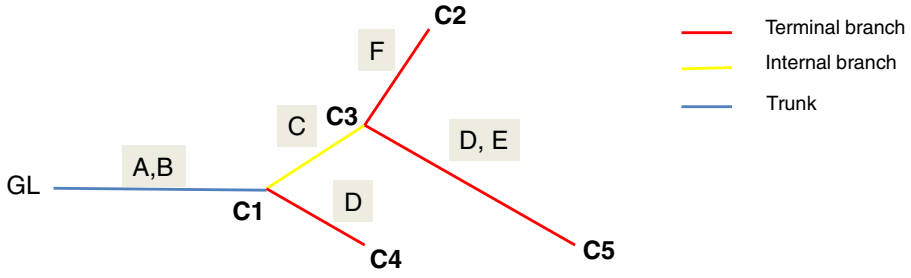

Fig. 1. An example of phylogenetic inference. The table indicates five clones C1–C5 which are characterised by the presence or the absence of mutations A, B, C, D, E, and F. Trees are rooted at the germline (GL). Mutations acquired by particular clones are indicated. Branch and trunk lengths are proportional to the number of mutations on the corresponding branch or trunk, which in this case is either 1 or 2. It is assumed that mutations cannot be lost which is why C4 is derived from C1 and not C2 or C3.

during therapy and therefore the knowledge of subclonal architecture is critical for prediction of therapeutic response. Early/clonal events are appealing therapeutic targets as they characterise the entire tumour population. Conversely, when subclonal mutations are targeted on their own, a suboptimal or even an adverse outcome can be expected. For example, *BRAF* mutations are frequently subclonal in multiple myeloma [14–17]. Treatment of BRAF wild-type myeloma cells with BRAF inhibitors caused paradoxical activation of the mitogen-activated protein kinase (MAPK) pathway, and this effect was further exaggerated in the presence of concurrent mutations in *NRAS* or *KRAS*[14]. Subclonal mutations in *NRAS* account for BRAF-inhibitor resistance in *BRAF*-mutant melanoma [18], and furthermore, BRAF inhibitors can induce metastases in *RAS*-mutant melanoma cells [19]. In patients with occult *RAS*-mutant cancers BRAF inhibitors have been reported to potentiate tumourigenesis (including *RAS*-mutant skin tumours, *RAS*-mutant leukaemia, and metastatic recurrence of *RAS*-mutant colorectal cancer) [20]. Subclonal amplification of *MET* and secondary *EGFR* mutations are linked to acquired resistance to EGFR inhibitors in EGFR-mutant lung cancer [21]. Thus, subclonal events can predict the mechanism of resistance to therapies that solely target the truncal driver mutation. An improved understanding of mutational timing could aid the design of combined therapeutic approaches or second line therapies. Finally, the view that truncal events are the most important therapeutic targets may be too simplistic. There is evidence that subclones can drive tumours by inducing tumour-promoting microenvironmental changes [22] and enhancing the tumourgenicity of the tumour as a whole [23]. Such subclones could provide additional targets for therapeutic intervention.

Mutational chronology is also relevant to our understanding of metastases and selective bottlenecks are again expected in this context due to differing environmental conditions between the primary and metastatic sites, and between metastatic sites. There is evidence that subclonal lineages become enriched in the metastases [24] and that different subclones in the primary give rise to inter-metastatic heterogeneity [25]. Analysis of four spatially separate metastases in two melanoma cases highlighted the genetic alterations responsible for differential drug resistance among metastatic tumours [26]. Thus, some subclones which occur later in tumour evolution could predict the composition of the metastases and reveal metastases-associated mutations.

Inferring mutational timing across the genomic landscape of a cancer can also estimate tumour age [27,28]. This knowledge could inform optimal cancer screening strategies by highlighting the window of opportunity for screening. Measures of clonal diversity can predict progression of Barrett's oesophagus to a full-blown adenocarcinoma [11]. The same group have reported that clonal diversity is modulated by the use of non-steroidal anti-inflammatories [29], suggesting a potential mechanism of chemoprevention by aspirin.

Despite these critical notions, to date, fairly limited evolutionary perspective featured in the research of cancer progression and treatment resistance. A recent study reported that of almost 7000 papers on therapeutic resistance and/or relapse in cancers only 1% had evolutionary terms in their abstracts [30]. Thus, the study of tumour evolution and mutational timing is an unrealised opportunity for advances in cancer research [30]. In this review we focus on recent strategies to infer mutational timing from next generation sequencing data, with a special focus on mutations in known driver genes.

2. Sampling methods

2.1. Paired samples

Most genomic datasets contain single data points from multiple individuals, but mutational timing can be inferred with greater accuracy from multiple either spatially or temporally separate samples from the same individual (Fig. 2A&B). To date, such studies have generally used manual curation to reconstruct phylogenetic trees. Mutational timing is inferred on the basis of mutations being shared (early events) or private (later events). Phylogenies are further resolved by incorporating computationally-estimated variant allele frequencies (VAFs), combined with local copy number states and estimations of tumour purity to identify sets of mutations whose VAFs shift together over time.

Spatial or geographical heterogeneity has been explored though multi-regional sampling of primary and/or metastatic tumours. This approach has been applied to a small number of tumour types including ccRCC [5,31], glioblastoma [32] lung adenocarcinoma [10,27] and breast cancer [33]. In ccRCC, driver mutations map mostly to the branches with only one or two driver mutations occurring on the trunk. By comparison, in glioblastoma more driver mutations mapped to the trunk, and in the two studies of lung adenocarcinoma, the majority of known driver mutations were classed as early events. These results should be interpreted with caution however, as our knowledge of driver genes is biased by frequent detection of events which are consistently clonal and detected at high frequency in single biopsy samples.

Studies of temporally separated samples have included matched primary-metastasis pairs [25,26,28,34–43], primary tumour and relapsed pairs [17,44,45] or serial tumour sampling [9,15,41,42]. These studies have shown that subclonal driver mutations anticipate the dominant genetic composition of the relapsing tumour in CLL [9]; that multiple subclones contribute to metastases in pancreatic cancer, prostate cancer and melanoma [26,28,46], and that phylogenetic trees across metastases can show organ-specific branches [25]. Conversely, in a single case report ccRCC metastases appeared to be monoclonal [38]. Some studies of matched primary-metastasis pairs found evidence of *de novo* mutations in the metastases [28,34,37,41,43]. Such mutations are either very late events and indicate ongoing tumour evolution in metastatic sites, or are present in minor subclones of the primary tumour which evaded detection (Fig. 2A). Recently, longitudinal studies have included post-mortem sampling of spatially separate metastases [47], an approach that promises to yield a highly accurate portrayal of cancers' evolution and mutational timing.

Employing these labour-intensive, technically challenging sampling techniques still does not resolve clonal structures in full as illustrated by the finding of *intraregional* subclonal populations [10]. Even very small regional biopsy samples contain an admixture of cells, which require deconvoluting. Further, the accuracy of clonal inferences is dependent on sequencing coverage, tumour purity and local copy number dynamics; inadequate depth of sequencing can mis-categorise shared mutations as private or ubiquitous [10]. Lastly, even multi-region sampling of a tumour represents a relatively small proportion of the overall tumour volume and could ignore significant clonal dynamics.

Ultimately, sequencing single cells or single tumour nuclei is the least biased approach to assessing heterogeneity within a tumour [48]. Single-cell genomic studies have been reported in a number of tumour types [49–53] revealing detailed clonal evolution and marked intercellular heterogeneity. Whilst promising, these technologies still require optimisation and presently they are not high throughput or widely available, nor can they be considered exhaustive approaches to define tumour phylogenies. In the future their use may become routine, but bulk sequencing will remain the basis of cancer evolution studies for the time being.

2.2. Single samples

Most genomic studies involve only single samples representing individual tumours. In these unfractionated samples mutational timing must be inferred from the frequency of each mutation in the population. Clonal/early mutations are supported by high VAFs and subclonal/late mutations by low VAFs. Taking into account tumour purity and local copy number, mutations with similar VAFs are clustered together into (sub)clones. In addition to SNVs and small insertions and deletion most studies consider copy number alterations (CNAs), although this is complicated by the absence of a digital readout for CNAs (in contrast

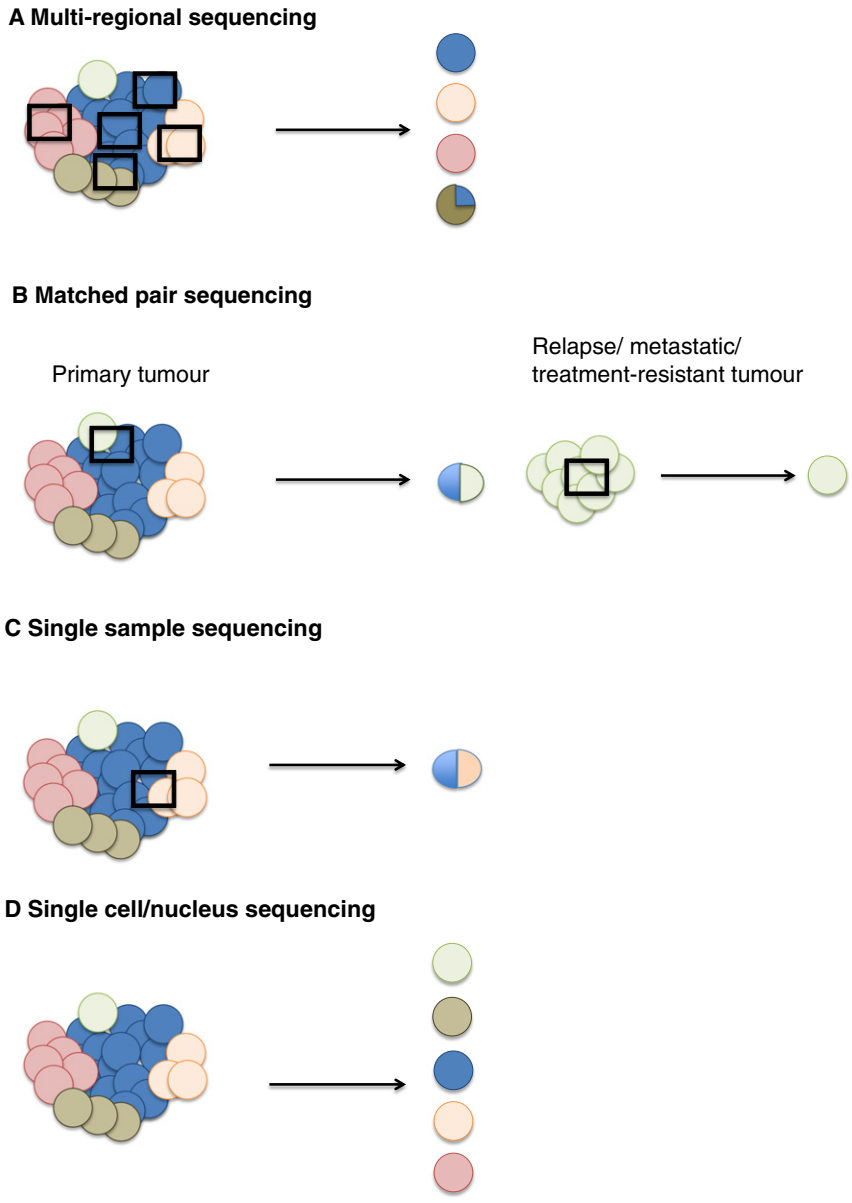

Fig. 2. Dissecting intratumour heterogeneity using different sampling approaches. Each colour represents a distinct subclone in the tumour mass. A. In this case multi-regional sequencing picks up the blue, orange and red clones as well as a mixture of blue and dark green clones. The light green clone is missed. B. Single biopsy of the primary tumour picks up a mixture of blue and light green clones. Paired sample shows clonal expansion of the light green clone. C. Single biopsy approach picks up a mixture of blue and orange clones only. D. All the clones are picked up by single cell sequencing.

to SNVs) [54]. There are a number of difficulties associated with this approach. First, the VAF of a given mutation is a composite measure of the proportion of tumour cells that harbour the mutation, the proportion of "normal" cells in the sample, and the number of allelic copies of the mutation in each cell. These factors can be partly deconvolved, but not completely resolved in bulk sequencing experiments. Second, it has been noted that single biopsy studies can misclassify subclonal mutations as clonal giving an illusion of clonal dominance [31]. Third, as sequencing reads are short, VAFs of different mutations have to be measured independently. Mutations with similar VAFs are assumed to co-occur within the same cells, but this may not be the case. Similarly, whilst it is assumed that clones at higher frequencies give rise to the clones with lower frequencies, subclones with overlapping VAFs cannot be distinguished [55]. Lastly, most mutational calling algorithms are not designed to uncover low frequency events, and so late-occurring mutations may be missed. Approaches such as MuTect [56], developed with the aim of detecting low-allele fractions represent a substantial advance, but in the absence of repeat or parallel sampling subclonal architecture will remain incompletely resolved.

A more general strategy to infer mutational timing, originally described by Pleasance et al. [57] is combining mutational data with chromosome or whole genome duplication events: mutations which precede chromosomal duplication show higher copy number than those occurring after duplication [58,59].

3. Computational methods

Various computational methods aimed at resolving clonal architectures have been published in recent years (for an in-depth review see, Beerenwinkel et al. 2015 [60]). The majority of methods seek to deconvolve the number of clonal and subclonal clusters. This problem is suited to a flexible Bayesian analysis, as mutations will be derived from an unknown number of subclones, present at unknown cancer cell fraction, and representing an unknown proportion of the entire

mutational catalogue [61]. Based on the identified SNV clusters, phylogenetic tree reconstruction can be performed. Most approaches make the assumption that the same mutation only occurs once in the process of a tumour's evolution and that no mutation can be lost. In the analysis of single samples there are often multiple trees which can fit the given pattern of VAFs, and few topological constraints exist to limit the space of solutions [60]. The advantage of analysing paired samples is that they contain different sets of VAFs and this can constrain the evolution space.

Below we describe published approaches to clonal inference and phylogenetic tree reconstruction based on single nucleotide variant (SNV) and allele specific copy number calls in single and multiple matched tumour samples

3.1. PyClone

PyClone [62] pioneered the integration of VAFs with allele specific copy number and purity estimates in order to define the subclonal composition of individual biopsies. The method was first applied to 104 triple negative breast cancers, shedding light on mutation timing of driver genes in these cancers (see below). Pyclone uses a Bayesian Dirichlet clustering process to jointly group deeply sequenced (>1000×) mutations and infer posterior density estimates over the cancer cell fraction (CCF) for each mutation. A limitation of this approach, however, is that it assumes all copy number events are clonal.

3.2. SciClone

SciClone [54] also applies a Bayesian clustering method to single or paired (spatially or temporally separate) samples to infer the subclonal composition of a tumour. A defining feature of SciClone's approach is that it focuses exclusively on SNV variants in copy-number neutral, loss of heterozygosity (LOH)-free portions of the genome. Although this feature circumvents issues associated with clonal and subclonal copy number aberrations it means that not all mutations can be associated with SNV clusters. Nevertheless, several studies using SciClone have been published to date [26,63], revealing subclonal structures in melanoma and leukaemia.

3.3. CloneHD

CloneHD [64] performs subclone reconstruction from either individual or integrated analysis of two types of data: copy number (derived from read depth and B-allele fraction in germline samples) and somatic SNVs using Hidden Markov Models. The algorithm can be applied to single as well as longitudinal or multiregional samples. Using CloneHD the authors deciphered clonal progression in CLL using WGS data alone [42], whilst still recovering an evolutionary history as was inferred by targeted deep sequencing in this study.

3.4. PhyloSub

PhyloSub [65] represents an extension of previous approaches in that it both clusters SNVs and performs phylogenetic tree inferences. In the case of multiple phylogenies being consistent with a given set of SNV frequencies, it represents the uncertainty in the tumour phylogeny using a “partial order plot”. As with other approaches PhyloSub's ability to identify subclones depends on their overall frequency in the population, the number of SNVs that define it, and the accuracy of the VAF estimate (which is in turn dependent on sequencing depth and accuracy of the copy number estimate). The authors apply the PhyloSub to a real dataset [42] and produce a good agreement with the semi-manually curated phylogenies in the published study. PhyloSub has recently been extended to include copy number aberrations and WGS data (PhyloWGS).

3.5. TrAp

TrAp [66] also attempts to define the evolutionary history of tumours. The method uses exome sequencing data and is specifically designed to deconvolute a single aggregate signal into its subclonal components. The authors' inference of the clonal evolution of AML cases using the TrAp algorithm was in agreement with that inferred by Ding et al. [44].

3.6. SubcloneSeeker

Based on identified SNV clusters, SubcloneSeeker [67] focuses on phylogenetic reconstruction. Specifically, SubcloneSeeker seeks to enumerate all possible evolutionary histories for a given tumour sample. The algorithm has been applied to a published study of clonal evolution in relapsed AML [44] producing mostly consistent subclone structures. The authors also present a *de novo* analysis of 15 matched primary-relapse pairs from the TCGA ovarian cancer dataset and identify two relapse patterns, which are underpinned by different order of clonal selection.

4. Mutational timing across cancer types

Broadly, there are two primary ways to infer mutation timing in relation to tumour evolution:

First, mutations and copy number events can be timed based on population frequencies. This approach assumes that common patterns can be identified in tumours from different patients [68,69] and can be informed by analysis of pre-malignant stages. For example, in Vogelstein and Fearon's seminal work a cross -section of data types was used to infer that colorectal cancer can largely be explained as a linear chain of four genetic events [70]. An extension of the linear view of tumour evolution is the oncogenetic tree approach, which represents tumour evolution as a tree structure, permitting diverging temporal ordering of events [71,72]. The tree structure can be further relaxed using tumour progression models [60] permitting insights into the order in which modification in signalling pathways occurs during tumourigenesis [73].

A second, alternative approach to infer mutation timing involves utilising the heterogeneity observed within single tumours. Such an approach uses phylogenetic reconstruction to distinguish between truncal events, occurring early in tumour evolution, and branched events, which necessarily occur after tumorigenesis. By analysing single cases this approach takes into account the variation in the evolution of individual cancers, although ultimately the combination of both approaches will gain maximal insights. In this section, we review the most up-to-date evidence for mutational timing using single tumour samples across different cancer types.

The results are summarised in Fig. 3.

4.1. Haematological malignancies

To date, the most comprehensive studies of clonal architecture have been applied to haematological malignancies. Compared to solid malignancies, analyses of such data are less hampered by extensive and varied stromal contamination. Haematological malignancies are characterised by a low mutation burden. Whilst this facilitates driver event identification it also means that fewer clonal markers are available for the reconstruction of tumour history, especially if only coding regions are included. For this reason whole genome sequencing (WGS) is the preferred method in leukaemia studies.

4.1.1. De novo and treatment-related acute myeloid leukaemia (AML)

Ding et al. [44] performed WGS of eight primary tumour/relapse AML pairs. Two distinct patterns of evolution were described in these

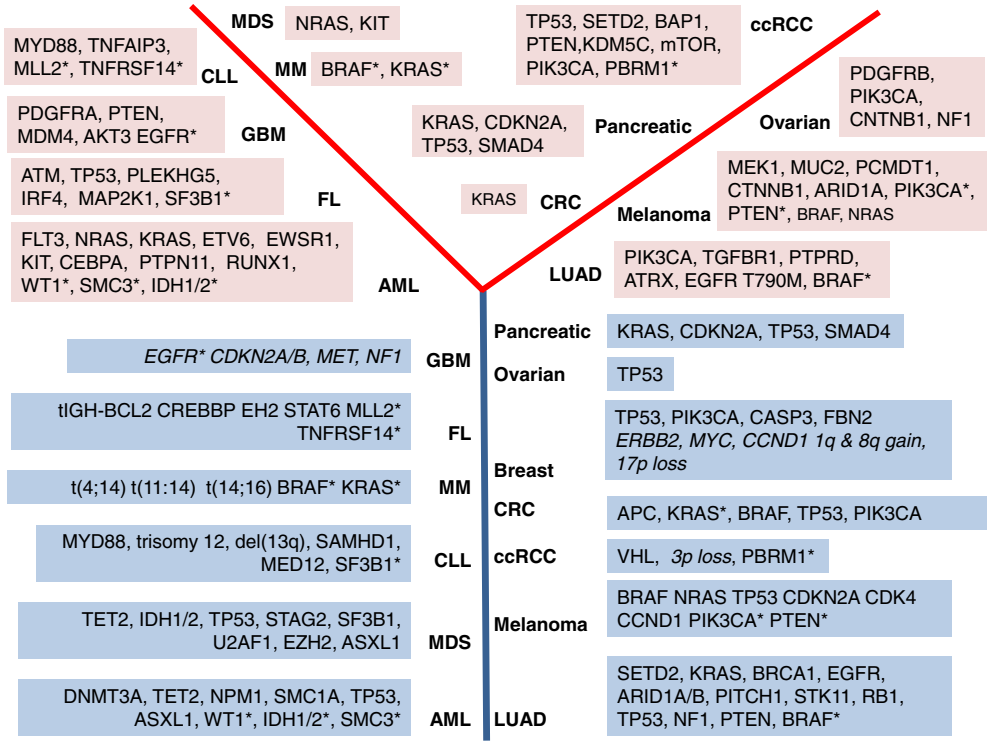

Fig. 3. Summary of clonal and subclonal mutations in specific tumour types. Clonal mutations are shown on the trunk (blue) and subclonal on the branches (red). Mutations which have been reported as both clonal and subclonal are indicated by an asterisk. Mutations which are predominantly clonal and infrequently detected as subclonal are shown in smaller font in the tree branches. Copy number events are shown in italics. AML = acute myeloid leukaemia; MDS = myelodysplastic syndrome; CLL = chronic lymphocytic leukaemia; LUAD = lung adenocarcinoma; ccRCC = clear cell renal cell carcinoma; GBM = gliobalstoma multiforme; CRC = colorectal cancer. The following studies are included in this data summary: Ding et al. 2012, Welch et al. 2012, Mazzarella et al. 2014, Klco et al. 2014, Walter et al. 2012, Wong et al. 201, Walter et al. 2012, Papaemmanuil et al. 2013, Landau et al. 2013, Schuh et al. 2012, Melchor et al. 2014, Bohn et al. 2014, De Bruin et al. 2014, Zhang et al. 2014, Su et al. 2012, Van Allen et al. 2014, Ding et al. 2014, Gerlinger et al. 2012, Gerlinger et al. 2014, Voss et al. 2014, Sankin et al. 2014, Francis et al. 2014, Szerlip et al. 2012, Diaz et al. 2012, Brannon et al. 2014, Donna et al. 2014, Nik-Zainal et al. 2012, Ding et al. 2010, Shah et al. 2009, TCGA 2011, Bashashati et al. 201, Campbell et al. 2012, and Yachida et al. 2010.

samples. Either the dominant clone in the primary tumour evolved into the relapse clone by gaining relapse-specific mutations, or, the more common pattern, a late subclone in the primary was enriched in the relapse sample. Mutations in *DNMT3A*, *NPMP*, *TPRT*, *SMC3*, *WT1 RUNX1* and *IDH2* were present at diagnosis and relapse and contributed to the founding clones, whilst mutations in *FLT3*, *IDH1* and *ETV6* were consistently subclonal. Other whole genome sequencing approaches to primary AML have identified exclusively clonal mutations in *DNMT3A*, *NPM*1, *IDH1* and *SMC1A* in a cohort of 24 cases, and predominantly subclonal mutations in *NRAS*, *FLT3*, *ETV6*, *EWSR1*[74,75]. In a meta-analysis of paired diagnosis-relapse samples which had been profiled by a variety of methods [76] either loss or gain of mutations at relapse was notable in *FLT3*, *KIT*, *NRAS/KRAS*, *WT*1 and *IDH1/2* suggesting that these events occur in subclones. Once more *DNMT3A*, *TET2* and *NMP1* were shown to be clonal events.

In keeping with these studies Klco et al. [77] described subclonal mutations in *FLT3* and *IDH1/2*. This study also incorporated xenotransplantation from unmanipulated tumour material. Different subclones had varying engraftment potential, (no founding clones were observed in the engrafting populations), but there was no direct relationship with the evolutionary history of leukaemia in the patient.

AML can arise from a pre-existing myelodysplastic syndrome (MDS) (secondary AML) and also as a consequence of previous chemotherapy (treatment-related AML). Walter et al. [78] reconstructed the clonal architecture of progression from MDS to AML. All seven cases under study conformed to the linear models of clonal evolution: MDS-founding clone persisted in secondary AML and was carried forward with each acquisition of new sets of mutations, resulting in an increasing mutational burden. Mutations in *TET2*, *IDH1/2*, *STAG2*, *TP53* and *U2AF1* were observed in both sets of samples indicating that they are early founding events in MDS. Mutations in *WT1*, *PTPN11*, *RUNX1*, *SMC3*, *FLT3*, *RAS* and *CEBPA* were restricted to the transformed AML sample suggesting they were later events. The same group confirmed their findings in an extension cohort of secondary AML cases [79]. Critically, in the latter study the authors compared the clonal architecture defined by WGS to that inferred from SNVs in 94 candidate genes concluding that the VAFs of candidate genes did not fully recapitulate the clonal architecture defined by WGS.

Therapy-related AML is characterised by a higher frequency of *TP53* mutations and resistance to chemotherapy. A recent study of therapy-related AML and MDS [80] detected very low frequency *TP53* mutations long before AML/MDS was diagnosed and in some cases before chemotherapy was given, suggesting that *TP53* mutations were not a direct result of treatment. Further, *TP53* mutations were detectable in healthy volunteers of older age [80]. These findings support a model in which rare haematopoietic progenitor cells harbour age-related *TP53* mutations which are resistant to chemotherapy and which expand preferentially following therapy. In two separate studies of WES of peripheral blood cells involving over 30, 000 healthy individuals clonal haematopoiesis was shown to increase with age [81,82]. Detectable clonal expansions involved the known early driver events in AML: *DNMT3A*, *ASXL1*, and *TET2* and were associated with increased risk of haematological cancers. In a subset of cases it was confirmed that AML arose from pre-existing clones [81]. Detection of these early events in the blood of healthy individuals presents a potential cancer screening opportunity.

Finally, a report of single cell sequencing from cases of MDS progressing to secondary AML [63] validated the clonal architecture

inferred from WGS of bulk tumour material, additionally resolving the clonality of a few initially ambiguous mutations.

4.1.2. Chronic lymphocytic leukaemia (CLL)

Landau et al. [9] performed an integrated analysis of whole exome sequencing and copy number variation in 149 cases of CLL. Using VAFs they identified events that were predominantly clonal/early, including *MYD88*, trisomy 12, and del 13q. Mutations in *ATM*, *TP53* and *SF3B1* were predominantly subclonal/late events. In a subset of patients they obtained temporally separate samples and demonstrated that disease progression coincided with the expansion of subclones containing driver mutations. In keeping with this observation, independent of the driver identity, subclonality of driver events was linked to adverse clinical outcomes in CLL.

Schuh et al. [42] used whole genome sequencing to track clonal architecture in three cases of CLL at five separate time points for up to seven years. Subclonal populations were evident at all time points. Clonal mutations were detected in genes recurrently mutated in CLL including *SF3B1*, *SAMHD1* and *MED12*. *ATM*, *PLEKHG5*, and *IRF4* were subclonal events. The patterns of progression varied in the three cases. In one instance disease progression was linked to the expansion of a subclone harbouring a mutation in MAP2K1 with ERK1/2 phosphorylation in lymphocytes mirroring the expansion. By contrast, in another case the balance of subclonal and clonal populations remained steady throughout the disease course suggesting that factors other than clonal evolution drove disease progression in this instance.

4.1.3. Myelodysplastic syndrome (MDS)

Walter et al. [79] screened 94 candidates (recurrently mutated genes) in a cohort of 150 cases of MDS. The range of VAFs was broad in all cases with no genes exclusively mutated early or late. However, they made several observations about mutational pairing. Mutations within a gene group including spliceosome, transcription factor, activated signalling/*RAS* pathway and cohesion were largely mutually exclusive. *TP53* mutations were stand-alone mutational events in two third of the cases but they co-occurred with complex karyotype. They observed co-occurrence of mutations in three pairs of genes, including *RUNX1* and *STAG2*, *EZH2* and *TET2*, and *BCOR* and *U2AF1* and a mutual exclusivity between *TP53* and spliceosome genes, and *NRAS* and cohesin genes.

Papaemmanuil et al. [83] also applied a targeted approach sequencing 111 genes across 738 cases of MDS. They present strong evidence that mutations in splicing factors including *U2AF1*, *SF3B1* and *U2AF1* as well as those in DNA methylation genes (*TET2*, *EZH2*, *ASXL1*) occur early in disease evolution, whilst mutations in kinases such as *NRAS* and *KIT* are late events. In contrast to CLL, driver mutations had equivalent prognostic significance, whether clonal or subclonal, and survival was linked to the overall number of driver mutations. Lastly, the authors infer that early driver mutations can dictate the future trajectories of disease evolution in MDS although longitudinal studies are required to confirm these findings [83].

4.1.4. Multiple myeloma (MM)

Several studies have reported whole genome [14,17], exome [15,16] and single cell sequencing [84] of MM samples. The only consistently early events are the characteristic translocations t(4;14), t(11:14) and t(14;16). Mutations in *BRAF* and *RAS* were observed as both clonal and subclonal, suggesting that these variants can contribute to tumour initiation, maintenance or progression. Unusually, *BRAF* and *RAS* mutations co-occurred in some of the samples [14,17]. In some cases both variants were present at a subclonal level and potentially drawn from different subpopulations but in one case both *BRAF* and *KRAS* mutations were found in the same (founding) clone. These observations are suggestive of parallel evolution, which has been reported in other cancers [5], highlighting the importance of the RAS-RAF pathway in MM. In keeping with this notion, a recent report linked the evolution of a $BRAF^{V600E}$ subclone as the mechanism of disease progression in a patient with MM [85].

4.1.5. Follicular lymphoma (FL)

Using CD20 as the marker Green et al. [86] sorted FL cells from 8 cases (including two matched FL-relapsed FL pairs). *IGH-BCL2* translocation and *CREBBP* mutations were clonal and maintained between diagnosis and relapse. Mutations in *MLL2* and *TNFRSF14* were subclonal and lost at relapse in one case. Interestingly, MLL2 is highly mutated in FL and this has been taken as evidence that it is also an early event. The findings in this study caution against using mutational frequency as a surrogate for clonality. Bodor et al. [87] analysed 101 *EZH2*-mutated cases of FL including 33 paired diagnosis-relapse samples and showed that *EZH2* mutations are predominantly clonal events. Okosun et al. [88] performed WES or WGS on ten cases of FL-transformed FL pairs, and reported clonally dominant mutations in *CREBBP*, *EZH2*, *STAT6*, but also *MLL2* and *TNFRSF14*, which were reported as subclonal in a previous study [86]. Mutations in *MYD88* and *TNFAIP3* were restricted to the transformation biopsy indicating that they are late events.

4.2. Solid malignancies

4.2.1. Lung adenocarcinoma (LUAD)

With respect to *EGFR* genotype both intratumour heterogeneity [89] and discordance in primary-metastasis pairs [90] have been reported in LUAD with only one study contradicting these findings [91]. These observations are in keeping with varied and mixed responses to EGFR inhibitors in *EGFR*-mutant LUADs. Further, variants associated with secondary resistance to EGFR inhibiton such as $EGFR^{T790M}$ and *MET* amplification are found in the subclones of the pretreatment tumour [21,92,93]. By contrast, secondary mutations in ALK which drive resistance to ALK inhibitors in ALK-rearranged tumours appear to arise *de novo* in progressing tumours [92].

On genome and exome-wide scale two recent studies have reported multi-region sequencing of primary Stage I–III LUAD tumours. Zhang et al. [10] applied multi-region whole-exome sequencing (WES) to 11 cases. They reveal evidence of ITH in all the cases but find that majority of mutations are ubiquitous. However, as LUADs are characterised by tobacco-induced high mutational burden, majority of clonal mutations are likely to be passengers acquired prior to malignant transformation. The size of the subclonal fraction correlated with patients' outcome in this small data set. Mutations in known cancer genes were early events (*SETD2*, *KRAS*, *BRCA1*, *EGFR*, *ARID1B*, *PITCH1*, *ARID1A* and *STK11*) with the exception of a mutation in *ATRX*, which was subclonal in one case. We [27] profiled multiple regions of seven cases of operable LUAD using a combination of WES and WGS. We too demonstrate that known driver events occur early in the course of lung carcinogenesis (*EGFR*, *RB1*, *TP53*, *NF1*, *SETD2*, *PTEN*). Mutations in *PIK3CA*, *TGFBR1* and *PTPRD* were subclonal/late events, whilst activating mutations in *BRAF* were reported as both clonal and subclonal. Compared to ccRCC tumours, LUADs appear to have less ITH, however there are important caveats to be considered in the comparison of these studies. First is that the LUAD cohorts [10,27] included earlier stage tumours than the ccRCC cohort [5], second, as alluded to above, many of the truncal events will have occurred pre-transformation and third, approaches to identifying driver events are based on overall mutational frequencies and by definition may omit drivers which are consistently subclonal.

4.2.2. Melanoma

Most sequencing studies, including the TCGA cohort, consist of metastatic melanoma tissue. Melanoma primary lesions are small and usually required in their entirety for histological analysis. In this respect, phylogenetic studies have been limited, but not uninformative. Ding et al. [26] inferred clonal structures from a combination of whole genome sequencing and targeted re-sequencing of 124 cases of melanoma. Applying SciClone to deeply sequenced calls they clustered

mutations with similar allelic fractions, although due to the high mutational burden and complex copy number landscape in melanoma the boundaries of some clusters were difficult to establish [26]. They observe that activating mutations in *BRAF* and *NRAS* are consistently early events in melanoma. This is congruous with their role in melanomagenesis: both *BRAF*[94] and *NRAS*[95] mutations are found in acquired naevi, which are precursors to melanoma. However, more recent studies challenge the notion that they are exclusively founder/clonal events. Lin et al. [96] isolated and sequenced single melanoma cells from primary melanomas and found they contained both *BRAF*-wild-type and *BRAF*-mutant tumour cells, observing that *BRAF*-mutant subclones were always selected during progression. Several groups have reported co-existing *NRAS* and *BRAF* mutant subclones within the same primary [97] or metastatic lesions [98]. This is at odds with the long-held view of their mutual exclusivity [99]. These recent finding illustrate that both *BRAF* and *NRAS* mutations *can* be subclonal/late events in melanoma which has special relevance for mechanisms of progression on BRAF inhibitors [100]. In patients with co-existent *BRAF* and *RAS* mutation BRAF inhibitors can have paradoxical, tumour-enhancing effects.

With the exception of *MEK1* mutations [100,101] components of the MAPK pathway are predominantly early events. It is known that loss of tumour suppressor genes including *PTEN*, *CDKN2A* and *TP53*, or cell-cycle regulation genes such as *CCND1* and *CDK4*[102] are needed for malignant transformation of *BRAF* mutant naevi. Therefore mutations in these gens occur after *BRAF* mutations although their exact timing is unclear. It is likely that mutations in *CDKN2A* and *TP53* occur early on, whilst components of the PI3K–AKT pathway were found to be both clonal and subclonal, and mutations in *MUC2*, *PCMDT1*, *CTNNB1*, and *ARID2* were mostly subclonal [103]. Subclonal expansion is linked to BRAF inhibitor resistance in *BRAF*-mutant melanomas [101], however resistance can also be mediated multiple clonal events in the RAS–RAF and the PI3K–AKT pathways.

4.2.3. Clear cell renal cell carcinoma (ccRCC)

Multi-region sequencing of 10 ccRCCs revealed that mutations in the von Hippel–Lindau (*VHL*) gene, together with the loss of chromosome 3p, are obligatory early events in this cancer type [5]whilst mutations in *PBRM1* could be either clonal and subclonal. However, mutations in *TP53*, *SETD2*, *BAP1*, *PTEN*, *mTOR*, *PIK3CA* and *KDM5C* were only ever found to be subclonal, suggesting that they occur later in tumour evolution. Preclinical studies have shown that BAP1 cooperates with VHL to induce ccRCC [104]. Another sequencing study focused on profiling five genes through multi-regional sequencing of ccRCC tumours [105]. In this case *VHL* mutations were consistently clonal, *PBRM1* was clonal in 60/% of cases and *SETD2* and *BAP1* in a third of cases and *KDM5C* in <25% of cases. This study sampled a maximum of four regions per tumour and under sampling may have given the illusion of clonality with respect to *BAP1*, *SETD2* and *KDM5C*. A further study [106] examined up to four regions of primary ccRCC tumours and demonstrated clonal mutations in *TP53* and *PBRM1* and subclonal mutations in *BAP1*. Both clonal and subconal mutations were detected in *TSC1* and *mTOR* and their presence was linked to extended benefit from mTOR inhibitors. As we found that *SETD2* mutations were a later event occurring in the proximal branches of the tumour phylogenetic trees [5] we hypothesised that SETD2 loss of function might drive ITH. Silencing SETD2 led to defective homologous recombination repair, DNA replication stress and impaired localisation of DNA polymerase delta to chromatin. Consistent with these observations, we found that breakpoint regions in *SETD2* mutant tumours are localised towards H3K36 sites that are normally trimethylated by SETD2. These data suggest that mutational timing can be used to infer novel drivers of branched evolution [107].

4.2.4. Glioblastoma multiforme (GBM)

GBM is an aggressive primary brain malignancy associated with extremely poor prognosis and limited response to systemic therapies. GBM samples are characterised by marked ITH with evidence of activation of multiple receptor tyrosine kinases within a single tumour [108,109]. However, due to sampling difficulties, detailed studies of clonal evolution in GBM have been limited. Tumours are often resected in a piecemeal fashion, which distorts their architecture, and compromises spatial sampling. Opportunities for temporal sampling are infrequent, as following primary resection majority of patients do not undergo repeat surgery. Sottoriva et al. [32] deployed an especially designed method to multi-region sample 11 cases of GBM. Each case displayed specific patterns of evolution, but overall, copy number alterations in *EGFR*, *CDKN2A/B/p14ARF*, *MET* and *NF1* were identified as early events, and aberrations in *PDGFRA*, *PTEN*, *MDM4*, and *AKT3* as late events.

Using a combination of single cell and bulk sequencing, Francis et al. [110] demonstrated that following EGFR amplification, parallel evolution involves multiple distinct EGFR variants. Study authors propose two models of clonal diversification in GBM: one in which common clonal mutations are followed by divergence of RTK genotype (*e.g.* EGFR, PDGFR and MET) [109] and the other where evolution is underpinned by the emergence of multiple variants of a single RTK (*e.g.* EGFR) [23,110].

4.2.5. Colorectal cancer (CRC)

The established model of colorectal tumourigenesis proceeds through a linear evolution starting with an inactivating mutation in *APC*, followed by activating mutations in *KRAS*/*BRAF*, and subsequent waves of clonal expansion driven by mutations in *TGFB*, *PIK3CA* and *TP53*[111]. This model is largely supported by genomic studies to date. Driver mutations in *KRAS*, *NRAS* and *BRAF* are concordant in primary-metastasis studies, implying that they are early events which persist throughout the disease course [112]. Recently, ultra-deep sequencing of 15 matched synchronous primary-metastasis pairs confirmed concordance with respect to *PIK3CA* and *TP53* genotypes [113]. Other studies have confirmed limited variation in primary-metastasis or inter-metastases comparisons [35,36]. One study reported several *de novo* alterations in the metastases, including potentially deleterious mutations in *FBXW7*, *DCLK1* and *FAT2*[35]. Genomic profiling of individual glands and single cells from 15 cases of CRC confirmed that mutations in *APC*, *KRAS PIK3CA* and *TP53* were early events [4].

Patients with metastatic CRC whose tumours are confirmed to be *RAS* wild-type (WT) are often treated with EGFR inhibitors. Following an initial response, patients invariable develop resistance to anti-EGFR therapy. In a study reported by Diaz et al. [114] almost 40% of patients with *KRAS* WT tumours treated with panitumumab, an EGFR inhibitor had *KRAS* mutations detectable in the circulating tumour DNA at the time of progression. Multiple distinct *KRAS* mutations were evident in three cases. Mathematical modelling performed in this study indicates that *KRAS* mutations were present in subclones prior to panitumumab treatment. Although this hypothesis was not confirmed by sequencing of pre-treatment tumours, it is in keeping with what has been observed in other cancers. Thus, it appears that *KRAS* mutations can also be late events in CRC and that in this capacity they drive acquired resistance to EGFR blockade.

4.2.6. Breast cancer

Several analyses of paired samples have reported on clonal evolution in breast cancer. Shah et al. [41] performed whole genome sequencing of a metastatic sample followed by profiling of select variants in the primary tumour, which was removed 9 years previously. 19 mutations were exclusive to the metastasis, suggesting they had arisen *de novo*. Mutations in *ABCB11*, *HAUS3*, *SLC24A4*, *SNX4* and *PALB2* were detected in the primary tumour with VAFs comparable to those in the metastasis, whilst mutations in *KIF1C*, *USP28*, *MYH8*, *MORC1*, *KIAA1468* and *RNASEH2A* were enriched in the metastasis. Ding et al. [24] reported whole exome sequencing of a trio of a primary breast tumour, brain metastasis and a xenograft derived from the primary tumour. Clonal mutations in *JAK2* and *CSMD1* had comparable VAFs in all three samples,

whilst mutations in *NRK*, *MAP3K8* and *PTPRJ* were significantly enriched in the metastasis. With the exception of a *TP53* mutation (which was only enriched in the xenograft), the mutation enrichment pattern in the xenograft mirrored that in the metastasis.

Nik-Zainal et al. [61] used a novel algorithm [115] to reconstruct the genomic history of 21 breast cancers. Multiple subclonal populations were detected with a dominant subclone evident in all cases. Mutations in *TP53*, *PIK3CA* and *ERBB2*, *MYC* and *CCND1* amplification, gains in 1q and 8q and losses of 17p were clonal, with subsequent divergence among subclones mostly evident in large scale chromosomal events.

Wang et al. [50] performed WGS/WES on ~50 single nuclei isolated from two cases of oestrogen-receptor positive breast cancer (ERBC) and triple-negative breast cancer (TNBC), respectively. Increased sampling led to detection of additional subclonal variants, but overall, TNBC was characterised by a greater extent of clonal diversity. They detected clonal mutations in known cancer genes including *PIK3CA*, *CASP3* and *FBN2*, and subclonal mutations in *TGFB2*, *CHRM5*, *AURKA*, *SYNE2* and *PP2R1*.

4.2.7. Ovarian cancer

TCGA analysis of ovarian carcinoma demonstrated that *TP53* mutations are present in almost all cases indicating their role as an early event in these tumours. Most other mutations were low frequency events mutated in <5% of all cases [116]. Bashshati et al. [40] performed a combined genomic analysis of six cases with multiple temporally and spatially separate samples revealing profoundly divergent mutational profiles and unique evolutionary trajectories in each case. *TP53* mutations were confirmed as consistently early/clonal events, but mutations in other driver genes, including, *PDGFRB*, *PIK3CA* and *CNTNB1* and *NF1* were mostly subclonal.

4.2.8. Pancreatic cancer

Two studies included 7 matched primary and metastases pairs and showed that mutations in *KRAS*, *CDKN2A*, *TP53* and *SMAD4* were consistently early events [25,28]. Many mutations across the 7 cases were subclonal, but there was only one recurrent subclonal event, a mutation in *OVCH1*, reported in two patients. Based on functionality, private mutations in *CNTN5*, *DOCK2*, *MEPIA* and *LMTK2* could be contributing to the metastatic process in these cases [28]. Data from these studies indicates ongoing evolution in the metastatic sites, suggesting that the repertoire of mutations required for metastatic progression is not fully contained in the primary tumour. Additionally, Campbell et al. show that phylogenetic trees across spatially separate metastases show organ-specific branches [25].

5. Conclusions

Mutations that occur early on in the disease course may be ideal therapeutic targets, whilst mutations that occur later are linked to disease progression and treatment resistance. Most studies to date have inferred mutational timing from single samples from multiple individuals. Current computational methods will require ongoing refinement in order to deal with the limitations of bulk unpaired-sample sequencing.

Paired sampling offers an improved resolution of mutational timing. Formalised longitudinal studies such as TRACERx [117] will attempt to map cancer's spatiotemporal diversity in great detail. Testing of *in vitro* and *in vivo* models derived from (multi)sampled tumours can be integrated with the genomic data to present functionally relevant subclonal dynamics. Ultimately, single cell sequencing will provide the most precise estimates of chronology of mutational events, but these techniques are far from widely accessible at this stage. Where tissue sampling is not practical circulating tumour DNA (ctDNA) can be used as a surrogate for tumour tissue. However, it remains to be seen whether analyses of ctDNA can fully recapitulate the diversity of the primary or metastatic tumours.

There is a striking variability within and across tumour types as to the timing of different mutations. At this stage, *VHL* mutations and loss of 3p in ccRCC and *APC* mutations in CRC appear to be the only obligatory truncal events. *TP53* shows fidelity within tumour types (early in ovarian cancer and late in ccRCC) but it is promiscuous from one cancer to the next. This may reflect its role in a diverse range of cellular processes, potentially as both a tumour suppressor and an oncogene, or even the underlying mutational processes: mutagen-induced *TP53* mutations are frequently early events [118]. *BRAF* mutations are associated with many malignancies but their founder mutation status appears to be limited to melanoma. Considering the evidence to date, a distinction emerges between haematological and solid malignancies. Mutations in RTKs are frequently early mutations in solid tumours whilst in leukaemia and multiple myeloma they are predominantly late events. These patterns could be the result of the different microenvironments, or the spatial constraints in solid tumours [119]. Thus, although the same genes can be altered in many different tumour types, the relative timing of their disruption with respect to tumour evolution is varied, indicating the importance of tissue context.

The ability to estimate mutational timing presents both prognostic and therapeutic opportunities. It is likely that mutational timing when combined with an analysis of distinct genomic instability patterns will generate biological opportunities to decipher drivers of branched evolution itself. Efforts to decipher "evolutionary rule books" across cancer types may support drug development and clinical trial design as well as inform drug discovery approaches to limit cancer diversity.

Conflict of interest

None.

Acknowledgements

S.T. is a Cancer Research UK Clinician Scientist (Grant No A18176). C.S. is a senior Cancer Research UK clinical research fellow and is funded by Cancer Research UK, the Rosetrees Trust, European Union Framework Programme 7 (projects PREDICT and RESPONSIFY, ID:259303), the Prostate Cancer Foundation, the European Research Council (10170) and the Breast Cancer Research Foundation.

References

[1] R.A. Burrell, N. McGranahan, J. Bartek, C. Swanton, The causes and consequences of genetic heterogeneity in cancer evolution, Nature 501 (2013) 338–345.
[2] L.R. Yates, P.J. Campbell, Evolution of the cancer genome, Nat. Rev. Genet. 13 (2012) 795–806.
[3] N.E. Navin, J. Hicks, Tracing the tumor lineage, Mol. Oncol. 4 (2010) 267–283.
[4] A. Sottoriva, H. Kang, Z. Ma, T.A. Graham, M.P. Salomon, J. Zhao, P. Marjoram, K. Siegmund, M.F. Press, D. Shibata, C. Curtis, A Big Bang model of human colorectal tumor growth, Nat. Genet. 47 (2015) 209–216.
[5] M. Gerlinger, S. Horswell, J. Larkin, A.J. Rowan, M.P. Salm, I. Varela, R. Fisher, N. McGranahan, N. Matthews, C.R. Santos, P. Martinez, B. Phillimore, S. Begum, A. Rabinowitz, B. Spencer-Dene, S. Gulati, P.A. Bates, G. Stamp, L. Pickering, M. Gore, D.L. Nicol, S. Hazell, P.A. Futreal, A. Stewart, C. Swanton, Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing, Nat. Genet. 46 (2014) 225–233.
[6] C. Tomasetti, B. Vogelstein, G. Parmigiani, Half or more of the somatic mutations in cancers of self-renewing tissues originate prior to tumor initiation, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 1999–2004.
[7] J.M. Weaver, C.S. Ross-Innes, N. Shannon, A.G. Lynch, T. Forshew, M. Barbera, M. Murtaza, C.A. Ong, P. Lao-Sirieix, M.J. Dunning, L. Smith, M.L. Smith, C.L. Anderson, B. Carvalho, M. O'Donovan, T.J. Underwood, A.P. May, N. Grehan, R. Hardwick, J. Davies, A. Oloumi, S. Aparicio, C. Caldas, M.D. Eldridge, P.A. Edwards, N. Rosenfeld, S. Tavare, R.C. Fitzgerald, O. Consortium, Ordering of mutations in preinvasive disease stages of esophageal carcinogenesis, Nat. Genet. 46 (2014) 837–843.
[8] Y. Sato, T. Yoshizato, Y. Shiraishi, S. Maekawa, Y. Okuno, T. Kamura, T. Shimamura, A. Sato-Otsubo, G. Nagae, H. Suzuki, Y. Nagata, K. Yoshida, A. Kon, Y. Suzuki, K. Chiba, H. Tanaka, A. Niida, A. Fujimoto, T. Tsunoda, T. Morikawa, D. Maeda, H. Kume, S. Sugano, M. Fukayama, H. Aburatani, M. Sanada, S. Miyano, Y. Homma, S. Ogawa, Integrated molecular analysis of clear-cell renal cell carcinoma, Nat. Genet. 45 (2013) 860–867.
[9] D.A. Landau, S.L. Carter, P. Stojanov, A. McKenna, K. Stevenson, M.S. Lawrence, C. Sougnez, C. Stewart, A. Sivachenko, L. Wang, Y. Wan, W. Zhang, S.A. Shukla, A. Vartanov, S.M. Fernandes, G. Saksena, K. Cibulskis, B. Tesar, S. Gabriel, N. Hacohen, M. Meyerson, E.S. Lander, D. Neuberg, J.R. Brown, G. Getz, C.J. Wu,

Evolution and impact of subclonal mutations in chronic lymphocytic leukemia, Cell 152 (2013) 714–726.
[10] J. Zhang, J. Fujimoto, J. Zhang, D.C. Wedge, X. Song, J. Zhang, S. Seth, C.W. Chow, Y. Cao, C. Gumbs, K.A. Gold, N. Kalhor, L. Little, H. Mahadeshwar, C. Moran, A. Protopopov, H. Sun, J. Tang, X. Wu, Y. Ye, W.N. William, J.J. Lee, J.V. Heymach, W.K. Hong, S. Swisher, P.A. Wistuba II, Futreal, intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing, Science 346 (2014) 256–259.
[11] C.C. Maley, A.K. Rustgi, Barrett's esophagus and its progression to adenocarcinoma, J. Natl. Compr. Cancer Netw. 4 (2006) 367–374.
[12] C.A. Ortmann, D.G. Kent, J. Nangalia, Y. Silber, D.C. Wedge, J. Grinfeld, E.J. Baxter, C.E. Massie, E. Papaemmanuil, S. Menon, A.L. Godfrey, D. Dimitropoulou, P. GuglielmelliI, B. Bellosillo, C. Besses, K. Dohner, C.N. Harrison, G.S. Vassiliou, A. Vannucchi, P.J. Campbell, A.R. Green, Effect of mutation order on myeloproliferative neoplasms, N. Engl. J. Med. 372 (2015) 601–612.
[13] A. Sottoriva, T. Graham, A Pan-cancer Signature of Neutral Tumor Evolution, 2015. bioRxiv.
[14] J.G. Lohr, P. Stojanov, S.L. Carter, P. Cruz-Gordillo, M.S. Lawrence, D. Auclair, C. Sougnez, B. Knoechel, J. Gould, G. Saksena, K. Cibulskis, A. McKenna, M.A. Chapman, R. Straussman, J. Levy, L.M. Perkins, J.J. Keats, S.E. Schumacher, M. Rosenberg, C. Multiple Myeloma Research, G. Getz, T.R. Golub, Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy, Cancer Cell 25 (2014) 91–101.
[15] J.J. Keats, M. Chesi, J.B. Egan, V.M. Garbitt, S.E. Palmer, E. Braggio, S. Van Wier, P.R. Blackburn, A.S. Baker, A. Dispenzieri, S. Kumar, S.V. Rajkumar, J.D. Carpten, M. Barrett, R. Fonseca, A.K. Stewart, P.L. Bergsagel, Clonal competition with alternating dominance in multiple myeloma, Blood 120 (2012) 1067–1076.
[16] J.B. Egan, C.X. Shi, W. Tembe, A. Christoforides, A. Kurdoglu, S. Sinari, S. Middha, Y. Asmann, J. Schmidt, E. Braggio, J.J. Keats, R. Fonseca, P.L. Bergsagel, D.W. Craig, J.D. Carpten, A.K. Stewart, Whole-genome sequencing of multiple myeloma from diagnosis to plasma cell leukemia reveals genomic initiating events, evolution, and clonal tides, Blood 120 (2012) 1060–1066.
[17] N. Bolli, H. Avet-Loiseau, D.C. Wedge, P. Van Loo, L.B. Alexandrov, I. Martincorena, K.J. Dawson, F. Iorio, S. Nik-Zainal, G.R. Bignell, J.W. Hinton, Y. Li, J.M. Tubio, S. McLaren, O.M. S, A.P. Butler, J.W. Teague, L. Mudie, E. Anderson, N. Rashid, Y.T. Tai, M.A. Shammas, A.S. Sperling, M. Fulciniti, P.G. Richardson, G. Parmigiani, F. Magrangeas, S. Minvielle, P. Moreau, M. Attal, T. Facon, P.A. Futreal, K.C. Anderson, P.J. Campbell, N.C. Munshi, Heterogeneity of genomic evolution and mutational profiles in multiple myeloma, Nat. Commun. 5 (2014) 2997.
[18] E. Romano, S. Pradervand, A. Paillusson, J. Weber, K. Harshman, K. Muehlethaler, D. Speiser, S. Peters, D. Rimoldi, O. Michielin, Identification of multiple mechanisms of resistance to vemurafenib in a patient with BRAFV600E-mutated cutaneous melanoma successfully rechallenged after progression, Clin. Cancer Res. 19 (2013) 5749–5757.
[19] B. Sanchez-Laorden, A. Viros, M.R. Girotti, M. Pedersen, G. Saturno, A. Zambon, D. Niculescu-Duvaz, S. Turajlic, A. Hayes, M. Gore, J. Larkin, P. Lorigan, M. Cook, C. Springer, R. Marais, BRAF inhibitors induce metastasis in RAS mutant or inhibitor-resistant melanoma cells by reactivating MEK and ERK signaling, Sci. Signal. 7 (2014) ra30.
[20] G.T. Gibney, J.L. Messina, I.V. Fedorenko, V.K. Sondak, K.S. Smalley, Paradoxical oncogenesis—the long-term effects of BRAF inhibition in melanoma, Nat. Rev. Clin. Oncol. 10 (2013) 390–399.
[21] A.B. Turke, K. Zejnullahu, Y.L. Wu, Y. Song, D. Dias-Santagata, E. Lifshits, L. Toschi, A. Rogers, T. Mok, L. Sequist, N.I. Lindeman, C. Murphy, S. Akhavanfard, B.Y. Yeap, Y. Xiao, M. Capelletti, A.J. Iafrate, C. Lee, J.G. Christensen, J.A. Engelman, P.A. Janne, Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC, Cancer Cell 17 (2010) 77–88.
[22] A. Marusyk, D.P. Tabassum, P.M. Altrock, V. Almendro, F. Michor, K. Polyak, Non-cell-autonomous driving of tumour growth supports sub-clonal heterogeneity, Nature 514 (2014) 54–58.
[23] M.M. Inda, R. Bonavia, A. Mukasa, Y. Narita, D.W. Sah, S. Vandenberg, C. Brennan, T.G. Johns, R. Bachoo, P. Hadwiger, P. Tan, R.A. Depinho, W. Cavenee, F. Furnari, Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma, Genes Dev. 24 (2010) 1731–1745.
[24] L. Ding, M.J. Ellis, S. Li, D.E. Larson, K. Chen, J.W. Wallis, C.C. Harris, M.D. McLellan, R.S. Fulton, L.L. Fulton, R.M. Abbott, J. Hoog, D.J. Dooling, D.C. Koboldt, H. Schmidt, J. Kalicki, Q. Zhang, L. Chen, L. Lin, M.C. Wendl, J.F. McMichael, V.J. Magrini, L. Cook, S.D. McGrath, T.L. Vickery, E. Appelbaum, K. Deschryver, S. Davies, T. Guintoli, L. Lin, R. Crowder, Y. Tao, J.E. Snider, S.M. Smith, A.F. Dukes, G.E. Sanderson, C.S. Pohl, K.D. Delehaunty, C.C. Fronick, K.A. Pape, J.S. Reed, J.S. Robinson, J.S. Hodges, W. Schierding, N.D. Dees, D. Shen, D.P. Locke, M.E. Wiechert, J.M. Eldred, J.B. Peck, B.J. Oberkfell, J.T. Lolofie, F. Du, A.E. Hawkins, M.D. O'Laughlin, K.E. Bernard, M. Cunningham, G. Elliott, M.D. Mason, D.M. Thompson Jr., J.L. Ivanovich, P.J. Goodfellow, C.M. Perou, G.M. Weinstock, R. Aft, M. Watson, T.J. Ley, R.K. Wilson, E.R. Mardis, Genome remodelling in a basal-like breast cancer metastasis and xenograft, Nature 464 (2010) 999–1005.
[25] P.J. Campbell, S. Yachida, L.J. Mudie, P.J. Stephens, E.D. Pleasance, L.A. Stebbings, L.A. Morsberger, C. Latimer, S. McLaren, M.L. Lin, D.J. McBride, I. Varela, S.A. Nik-Zainal, C. Leroy, M. Jia, A. Menzies, A.P. Butler, J.W. Teague, C.A. Griffin, J. Burton, H. Swerdlow, M.A. Quail, M.R. Stratton, C. Iacobuzio-Donahue, P.A. Futreal, The patterns and dynamics of genomic instability in metastatic pancreatic cancer, Nature 467 (2010) 1109–1113.
[26] L. Ding, M. Kim, K.L. Kanchi, N.D. Dees, C. Lu, M. Griffith, D. Fenstermacher, H. Sung, C.A. Miller, B. Goetz, M.C. Wendl, O. Griffith, L.A. Cornelius, G.P. Linette, J.F. McMichael, V.K. Sondak, R.C. Fields, T.J. Ley, J.J. Mule, R.K. Wilson, J.S. Weber, Clonal architectures and driver mutations in metastatic melanomas, PLoS ONE 9 (2014) e111153.
[27] E.C. de Bruin, N. McGranahan, R. Mitter, M. Salm, D.C. Wedge, L. Yates, M. Jamal-Hanjani, S. Shafi, N. Murugaesu, A.J. Rowan, E. Gronroos, M.A. Muhammad, S. Horswell, M. Gerlinger, I. Varela, D. Jones, J. Marshall, T. Voet, P. Van Loo, D.M. Rassl, R.C. Rintoul, S.M. Janes, S.M. Lee, M. Forster, T. Ahmad, D. Lawrence, M. Falzon, A. Capitanio, T.T. Harkins, C.C. Lee, W. Tom, E. Teefe, S.C. Chen, S. Begum, A. Rabinowitz, B. Phillimore, B. Spencer-Dene, G. Stamp, Z. Szallasi, N. Matthews, A. Stewart, P. Campbell, C. Swanton, Spatial and temporal diversity in genomic instability processes defines lung cancer evolution, Science 346 (2014) 251–256.
[28] S. Yachida, S. Jones, I. Bozic, T. Antal, R. Leary, B. Fu, M. Kamiyama, R.H. Hruban, J.R. Eshleman, M.A. Nowak, V.E. Velculescu, K.W. Kinzler, B. Vogelstein, C.A. Iacobuzio-Donahue, Distant metastasis occurs late during the genetic evolution of pancreatic cancer, Nature 467 (2010) 1114–1117.
[29] R.L. Kostadinov, M.K. Kuhner, X. Li, C.A. Sanchez, P.C. Galipeau, T.G. Paulson, C.L. Sather, A. Srivastava, R.D. Odze, P.L. Blount, T.L. Vaughan, B.J. Reid, C.C. Maley, NSAIDs modulate clonal evolution in Barrett's esophagus, PLoS Genet. 9 (2013) e1003553.
[30] C.A. Aktipis, V.S. Kwan, K.A. Johnson, S.L. Neuberg, C.C. Maley, Overlooking evolution: a systematic analysis of cancer relapse and therapeutic resistance research, PLoS ONE 6 (2011) e26100.
[31] M. Gerlinger, A.J. Rowan, S. Horswell, J. Larkin, D. Endesfelder, E. Gronroos, P. Martinez, N. Matthews, A. Stewart, P. Tarpey, I. Varela, B. Phillimore, S. Begum, N.Q. McDonald, A. Butler, D. Jones, K. Raine, C. Latimer, C.R. Santos, M. Nohadani, A.C. Eklund, B. Spencer-Dene, G. Clark, L. Pickering, G. Stamp, M. Gore, Z. Szallasi, J. Downward, P.A. Futreal, C. Swanton, Intratumor heterogeneity and branched evolution revealed by multiregion sequencing, N. Engl. J. Med. 366 (2012) 883–892.
[32] A. Sottoriva, I. Spiteri, S.G. Piccirillo, A. Touloumis, V.P. Collins, J.C. Marioni, C. Curtis, C. Watts, S. Tavare, Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 4009–4014.
[33] H. Zare, J. Wang, A. Hu, K. Weber, J. Smith, D. Nickerson, C. Song, D. Witten, C.A. Blau, W.S. Noble, Inferring clonal composition from multiple sections of a breast cancer, PLoS Comput. Biol. 10 (2014) e1003703.
[34] S. Turajlic, S.J. Furney, M.B. Lambros, C. Mitsopoulos, I. Kozarewa, F.C. Geyer, A. Mackay, J. Hakas, M. Zvelebil, C.J. Lord, A. Ashworth, M. Thomas, G. Stamp, J. Larkin, J.S. Reis-Filho, R. Marais, Whole genome sequencing of matched primary and metastatic acral melanomas, Genome Res. 22 (2012) 196–207.
[35] T. Xie, Y.B. Cho, K. Wang, D. Huang, H.K. Hong, Y.L. Choi, Y.H. Ko, D.H. Nam, J. Jin, H. Yang, J. Fernandez, S. Deng, P.A. Rejto, W.Y. Lee, M. Mao, Patterns of somatic alterations between matched primary and metastatic colorectal tumors characterized by whole-genome sequencing, Genomics 104 (2014) 234–241.
[36] S.Y. Lee, F. Haq, D. Kim, C. Jun, H.J. Jo, S.M. Ahn, W.S. Lee, Comparative genomic analysis of primary and synchronous metastatic colorectal cancers, PLoS ONE 9 (2014) e90459.
[37] L. Ouyang, J. Lee, C.K. Park, M. Mao, Y. Shi, Z. Gong, H. Zheng, Y. Li, Y. Zhao, G. Wang, H. Fu, J. Kim, H.Y. Lim, Whole-genome sequencing of matched primary and metastatic hepatocellular carcinomas, BMC Med. Genet. 7 (2014) 2.
[38] Y. Huang, S. Gao, S. Wu, P. Song, X. Sun, X. Hu, S. Zhang, Y. Yu, J. Zhu, C. Li, Z. Qin, L. Xie, Q. Yao, A. Tang, Z. Li, G. Guo, S. Wan, P. Dong, L. Sun, W. Li, D. Wang, Y. Gui, H. Yang, F. Zhou, X. Zhang, Z. Cai, Multilayered molecular profiling supported the monoclonal origin of metastatic renal cell carcinoma, Int. J. Cancer 135 (2014) 78–87.
[39] L. De Mattos-Arruda, F.C. Bidard, H.H. Won, J. Cortes, C.K. Ng, V. Peg, P. Nuciforo, A.A. Jungbluth, B. Weigelt, M.F. Berger, J. Seoane, J.S. Reis-Filho, Establishing the origin of metastatic deposits in the setting of multiple primary malignancies: the role of massively parallel sequencing, Mol. Oncol. 8 (2014) 150–158.
[40] A. Bashashati, G. Ha, A. Tone, J. Ding, L.M. Prentice, A. Roth, J. Rosner, K. Shumansky, S. Kalloger, J. Senz, W. Yang, M. McConechy, N. Melnyk, M. Anglesio, M.T. Luk, K. Tse, T. Zeng, R. Moore, Y. Zhao, M.A. Marra, B. Gilks, S. Yip, D.G. Huntsman, J.N. McAlpine, S.P. Shah, Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling, J. Pathol. 231 (2013) 21–34.
[41] S.P. Shah, R.D. Morin, J. Khattra, L. Prentice, T. Pugh, A. Burleigh, A. Delaney, K. Gelmon, R. Guliany, J. Senz, C. Steidl, R.A. Holt, S. Jones, M. Sun, G. Leung, R. Moore, T. Severson, G.A. Taylor, A.E. Teschendorff, K. Tse, G. Turashvili, R. Varhol, R.L. Warren, P. Watson, Y. Zhao, C. Caldas, D. Huntsman, M. Hirst, M.A. Marra, S. Aparicio, Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution, Nature 461 (2009) 809–813.
[42] A. Schuh, J. Becq, S. Humphray, A. Alexa, A. Burns, R. Clifford, S.M. Feller, R. Grocock, S. Henderson, I. Khrebtukova, Z. Kingsbury, S. Luo, D. McBride, L. Murray, T. Menju, A. Timbs, M. Ross, J. Taylor, D. Bentley, Monitoring chronic lymphocytic leukemia progression by whole genome sequencing reveals heterogeneous clonal evolution patterns, Blood 120 (2012) 4191–4196.
[43] X. Wu, P.A. Northcott, A. Dubuc, A.J. Dupuy, D.J. Shih, H. Witt, S. Croul, E. Bouffet, D.W. Fults, C.G. Eberhart, L. Garzia, T. Van Meter, D. Zagzag, N. Jabado, J. Schwartzentruber, J. Majewski, T.E. Scheetz, S.M. Pfister, A. Korshunov, X.N. Li, S.W. Scherer, Y.J. Cho, K. Akagi, T.J. MacDonald, J. Koster, M.G. McCabe, A.L. Sarver, V.P. Collins, W.A. Weiss, D.A. Largaespada, L.S. Collier, M.D. Taylor, Clonal selection drives genetic divergence of metastatic medulloblastoma, Nature 482 (2012) 529–533.
[44] L. Ding, T.J. Ley, D.E. Larson, C.A. Miller, D.C. Koboldt, J.S. Welch, J.K. Ritchey, M.A. Young, T. Lamprecht, M.D. McLellan, J.F. McMichael, J.W. Wallis, C. Lu, D. Shen, C.C. Harris, D.J. Dooling, R.S. Fulton, L.L. Fulton, K. Chen, H. Schmidt, J. Kalicki-Veizer, V.J. Magrini, L. Cook, S.D. McGrath, T.L. Vickery, M.C. Wendl, S. Heath, M.A. Watson, D.C. Link, M.H. Tomasson, W.D. Shannon, J.E. Payton, S. Kulkarni, P. Westervelt, M.J. Walter, T.A. Graubert, E.R. Mardis, R.K. Wilson, J.F. DiPersio, Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing, Nature 481 (2012) 506–510.

[45] B.E. Johnson, T. Mazor, C. Hong, M. Barnes, K. Aihara, C.Y. McLean, S.D. Fouse, S. Yamamoto, H. Ueda, K. Tatsuno, S. Asthana, L.E. Jalbert, S.J. Nelson, A.W. Bollen, W.C. Gustafson, E. Charron, W.A. Weiss, I.V. Smirnov, J.S. Song, A.B. Olshen, S. Cha, Y. Zhao, R.A. Moore, A.J. Mungall, S.J. Jones, M. Hirst, M.A. Marra, N. Saito, H. Aburatani, A. Mukasa, M.S. Berger, S.M. Chang, B.S. Taylor, J.F. Costello, Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma, Science 343 (2014) 189–193.
[46] M.C. Haffner, T. Mosbruger, D.M. Esopi, H. Fedor, C.M. Heaphy, D.A. Walker, N. Adejola, M. Gurel, J. Hicks, A.K. Meeker, M.K. Halushka, J.W. Simons, W.B. Isaacs, A.M. De Marzo, W.G. Nelson, S. Yegnasubramanian, Tracking the clonal origin of lethal prostate cancer, J. Clin. Invest. 123 (2013) 4918–4922.
[47] D. Juric, P. Castel, M. Griffith, O.L. Griffith, H.H. Won, H. Ellis, S.H. Ebbesen, B.J. Ainscough, A. Ramu, G. Iyer, R.H. Shah, T. Huynh, M. Mino-Kenudson, D. Sgroi, S. Isakoff, A. Thabet, L. Elamine, D.B. Solit, S.W. Lowe, C. Quadt, M. Peters, A. Derti, R. Schegel, A. Huang, E.R. Mardis, M.F. Berger, J. Baselga, M. Scaltriti, Convergent loss of PTEN leads to clinical resistance to a PI(3)Kalpha inhibitor, Nature 518 (2015) 240–244.
[48] E. Shapiro, T. Biezuner, S. Linnarsson, Single-cell sequencing-based technologies will revolutionize whole-organism science, Nat. Rev. Genet. 14 (2013) 618–630.
[49] X. Xu, Y. Hou, X. Yin, L. Bao, A. Tang, L. Song, F. Li, S. Tsang, K. Wu, H. Wu, W. He, L. Zeng, M. Xing, R. Wu, H. Jiang, X. Liu, D. Cao, G. Guo, X. Hu, Y. Gui, Z. Li, W. Xie, X. Sun, M. Shi, Z. Cai, B. Wang, M. Zhong, J. Li, Z. Lu, N. Gu, X. Zhang, L. Goodman, L. Bolund, J. Wang, H. Yang, K. Kristiansen, M. Dean, Y. Li, J. Wang, Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor, Cell 148 (2012) 886–895.
[50] Y. Wang, J. Waters, M.L. Leung, A. Unruh, W. Roh, X. Shi, K. Chen, P. Scheet, S. Vattathil, H. Liang, A. Multani, H. Zhang, R. Zhao, F. Michor, F. Meric-Bernstam, N.E. Navin, Clonal evolution in breast cancer revealed by single nucleus genome sequencing, Nature 512 (2014) 155–160.
[51] N.E. Potter, L. Ermini, E. Papaemmanuil, G. Cazzaniga, G. Vijayaraghavan, I. Titley, A. Ford, P. Campbell, L. Kearney, M. Greaves, Single-cell mutational profiling and clonal phylogeny in cancer, Genome Res. 23 (2013) 2115–2125.
[52] N. Navin, J. Kendall, J. Troge, P. Andrews, L. Rodgers, J. McIndoo, K. Cook, A. Stepansky, D. Levy, D. Esposito, L. Muthuswamy, A. Krasnitz, W.R. McCombie, J. Hicks, M. Wigler, Tumour evolution inferred by single-cell sequencing, Nature 472 (2011) 90–94.
[53] Y. Hou, L. Song, P. Zhu, B. Zhang, Y. Tao, X. Xu, F. Li, K. Wu, J. Liang, D. Shao, H. Wu, X. Ye, C. Ye, R. Wu, M. Jian, Y. Chen, W. Xie, R. Zhang, L. Chen, X. Liu, X. Yao, H. Zheng, C. Yu, Q. Li, Z. Gong, M. Mao, X. Yang, L. Yang, J. Li, W. Wang, Z. Lu, N. Gu, G. Laurie, L. Bolund, K. Kristiansen, J. Wang, H. Yang, Y. Li, X. Zhang, J. Wang, Single-cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm, Cell 148 (2012) 873–885.
[54] C.A. Miller, B.S. White, N.D. Dees, M. Griffith, J.S. Welch, O.L. Griffith, R. Vij, M.H. Tomasson, T.A. Graubert, M.J. Walter, M.J. Ellis, W. Schierding, J.F. DiPersio, T.J. Ley, E.R. Mardis, R.K. Wilson, L. Ding, SciClone: inferring clonal architecture and tracking the spatial and temporal patterns of tumor evolution, PLoS Comput. Biol. 10 (2014) e1003665.
[55] I. Hajirasouliha, A. Mahmoody, B.J. Raphael, A combinatorial approach for analyzing intra-tumor heterogeneity from high-throughput sequencing data, Bioinformatics 30 (2014) i78–i86.
[56] K. Cibulskis, M.S. Lawrence, S.L. Carter, A. Sivachenko, D. Jaffe, C. Sougnez, S. Gabriel, M. Meyerson, E.S. Lander, G. Getz, Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples, Nat. Biotechnol. 31 (2013) 213–219.
[57] E.D. Pleasance, R.K. Cheetham, P.J. Stephens, D.J. McBride, S.J. Humphray, C.D. Greenman, I. Varela, M.L. Lin, G.R. Ordonez, G.R. Bignell, K. Ye, J. Alipaz, M.J. Bauer, D. Beare, A. Butler, R.J. Carter, L. Chen, A.J. Cox, S. Edkins, P.I. Kokko-Gonzales, N.A. Gormley, R.J. Grocock, C.D. Haudenschild, M.M. Hims, T. James, M. Jia, Z. Kingsbury, C. Leroy, J. Marshall, A. Menzies, L.J. Mudie, Z. Ning, T. Royce, O.B. Schulz-Trieglaff, A. Spiridou, L.A. Stebbings, L. Szajkowski, J. Teague, D. Williamson, L. Chin, M.T. Ross, P.J. Campbell, D.R. Bentley, P.A. Futreal, M.R. Stratton, A comprehensive catalogue of somatic mutations from a human cancer genome, Nature 463 (2010) 191–196.
[58] S. Durinck, C. Ho, N.J. Wang, W. Liao, L.R. Jakkula, E.A. Collisson, J. Pons, S.W. Chan, E.T. Lam, C. Chu, K. Park, S.W. Hong, J.S. Hur, N. Huh, I.M. Neuhaus, S.S. Yu, R.C. Grekin, T.M. Mauro, J.E. Cleaver, P.Y. Kwok, P.E. LeBoit, G. Getz, K. Cibulskis, J.C. Aster, H. Huang, E. Purdom, J. Li, L. Bolund, S.T. Arron, J.W. Gray, P.T. Spellman, R.J. Cho, Temporal dissection of tumorigenesis in primary cancers, Cancer Discov. 1 (2011) 137–143.
[59] S.M. Dewhurst, N. McGranahan, R.A. Burrell, A.J. Rowan, E. Gronroos, D. Endesfelder, T. Joshi, D. Mouradov, P. Gibbs, R.L. Ward, N.J. Hawkins, Z. Szallasi, O.M. Sieber, C. Swanton, Tolerance of whole-genome doubling propagates chromosomal instability and accelerates cancer genome evolution, Cancer Discov. 4 (2014) 175–185.
[60] N. Beerenwinkel, R.F. Schwarz, M. Gerstung, F. Markowetz, Cancer evolution: mathematical models and computational inference, Syst. Biol. 64 (2015) e1–e25.
[61] S. Nik-Zainal, P. Van Loo, D.C. Wedge, L.B. Alexandrov, C.D. Greenman, K.W. Lau, K. Raine, D. Jones, J. Marshall, M. Ramakrishna, A. Shlien, S.L. Cooke, J. Hinton, A. Menzies, L.A. Stebbings, C. Leroy, M. Jia, R. Rance, L.J. Mudie, S.J. Gamble, P.J. Stephens, S. McLaren, P.S. Tarpey, E. Papaemmanuil, H.R. Davies, I. Varela, D.J. McBride, G.R. Bignell, K. Leung, A.P. Butler, J.W. Teague, S. Martin, G. Jonsson, O. Mariani, S. Boyault, P. Miron, A. Fatima, A. Langerod, S.A. Aparicio, A. Tutt, A.M. Sieuwerts, A. Borg, G. Thomas, A.V. Salomon, A.L. Richardson, A.L. Borresen-Dale, P.A. Futreal, M.R. Stratton, P.J. Campbell, C. Breast Cancer Working Group of the International Cancer Genome, The life history of 21 breast cancers, Cell 149 (2012) 994–1007.
[62] A. Roth, J. Khattra, D. Yap, A. Wan, E. Laks, J. Biele, G. Ha, S. Aparicio, A. Bouchard-Cote, S.P. Shah, PyClone: statistical inference of clonal population structure in cancer, Nat. Methods 11 (2014) 396–398.
[63] A.E. Hughes, V. Magrini, R. Demeter, C.A. Miller, R. Fulton, L.L. Fulton, W.C. Eades, K. Elliott, S. Heath, P. Westervelt, L. Ding, D.F. Conrad, B.S. White, J. Shao, D.C. Link, J.F. DiPersio, E.R. Mardis, R.K. Wilson, T.J. Ley, M.J. Walter, T.A. Graubert, Clonal architecture of secondary acute myeloid leukemia defined by single-cell sequencing, PLoS Genet. 10 (2014) e1004462.
[64] A. Fischer, I. Vazquez-Garcia, C.J. Illingworth, V. Mustonen, High-definition reconstruction of clonal composition in cancer, Cell Rep. 7 (2014) 1740–1752.
[65] W. Jiao, S. Vembu, A.G. Deshwar, L. Stein, Q. Morris, Inferring clonal evolution of tumors from single nucleotide somatic mutations, BMC Bioinformatics 15 (2014) 35.
[66] F. Strino, F. Parisi, M. Micsinai, Y. Kluger, TrAp: a tree approach for fingerprinting subclonal tumor composition, Nucleic Acids Res. 41 (2013) e165.
[67] Y. Qiao, A.R. Quinlan, A.A. Jazaeri, R.G. Verhaak, D.A. Wheeler, G.T. Marth, SubcloneSeeker: a computational framework for reconstructing tumor clone structure for cancer variant interpretation and prioritization, Genome Biol. 15 (2014) 443.
[68] M. Hoglund, A. Frigyesi, T. Sall, D. Gisselsson, F. Mitelman, Statistical behavior of complex cancer karyotypes, Genes Chromosomes Cancer 42 (2005) 327–341.
[69] C.S. Attolini, Y.K. Cheng, R. Beroukhim, G. Getz, O. Abdel-Wahab, R.L. Levine, I.K. Mellinghoff, F. Michor, A mathematical framework to determine the temporal sequence of somatic genetic events in cancer, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 17604–17609.
[70] E.R. Fearon, B. Vogelstein, A genetic model for colorectal tumorigenesis, Cell 61 (1990) 759–767.
[71] R. Desper, F. Jiang, O.P. Kallioniemi, H. Moch, C.H. Papadimitriou, A.A. Schaffer, Inferring tree models for oncogenesis from comparative genome hybridization data, J. Comput. Biol. 6 (1999) 37–51.
[72] N. Beerenwinkel, J. Rahnenfuhrer, M. Daumer, D. Hoffmann, R. Kaiser, J. Selbig, T. Lengauer, Learning multiple evolutionary pathways from cross-sectional data, J. Comput. Biol. 12 (2005) 584–598.
[73] Y.K. Cheng, R. Beroukhim, R.L. Levine, I.K. Mellinghoff, E.C. Holland, F. Michor, A mathematical methodology for determining the temporal order of pathway alterations arising during gliomagenesis, PLoS Comput. Biol. 8 (2012) e1002337.
[74] L. Mazzarella, L. Riva, L. Luzi, C. Ronchini, P.G. Pelicci, The genomic and epigenomic landscapes of AML, Semin. Hematol. 51 (2014) 259–272.
[75] J.S. Welch, T.J. Ley, D.C. Link, C.A. Miller, D.E. Larson, D.C. Koboldt, L.D. Wartman, T.L. Lamprecht, F. Liu, J. Xia, C. Kandoth, R.S. Fulton, M.D. McLellan, D.J. Dooling, J.W. Wallis, K. Chen, C.C. Harris, H.K. Schmidt, J.M. Kalicki-Veizer, C. Lu, Q. Zhang, L. Lin, M.D. O'Laughlin, J.F. McMichael, K.D. Delehaunty, L.A. Fulton, V.J. Magrini, S.D. McGrath, R.T. Demeter, T.L. Vickery, J. Hundal, L.L. Cook, G.W. Swift, J.P. Reed, P.A. Alldredge, T.N. Wylie, J.R. Walker, M.A. Watson, S.E. Heath, W.D. Shannon, N. Varghese, R. Nagarajan, J.E. Payton, J.D. Baty, S. Kulkarni, J.M. Klco, M.H. Tomasson, P. Westervelt, M.J. Walter, T.A. Graubert, J.F. DiPersio, L. Ding, E.R. Mardis, R.K. Wilson, The origin and evolution of mutations in acute myeloid leukemia, Cell 150 (2012) 264–278.
[76] J.S. Welch, Mutation position within evolutionary subclonal architecture in AML, Semin. Hematol. 51 (2014) 273–281.
[77] J.M. Klco, D.H. Spencer, C.A. Miller, M. Griffith, T.L. Lamprecht, M. O'Laughlin, C. Fronick, V. Magrini, R.T. Demeter, R.S. Fulton, W.C. Eades, D.C. Link, T.A. Graubert, M.J. Walter, E.R. Mardis, J.F. Dipersio, R.K. Wilson, T.J. Ley, Functional heterogeneity of genetically defined subclones in acute myeloid leukemia, Cancer Cell 25 (2014) 379–392.
[78] M.J. Walter, D. Shen, L. Ding, J. Shao, D.C. Koboldt, K. Chen, D.E. Larson, M.D. McLellan, D. Dooling, R. Abbott, R. Fulton, V. Magrini, H. Schmidt, J. Kalicki-Veizer, M. O'Laughlin, X. Fan, M. Grillot, S. Witowski, S. Heath, J.L. Frater, W. Eades, M. Tomasson, P. Westervelt, J.F. DiPersio, D.C. Link, E.R. Mardis, T.J. Ley, R.K. Wilson, T.A. Graubert, Clonal architecture of secondary acute myeloid leukemia, N. Engl. J. Med. 366 (2012) 1090–1098.
[79] M.J. Walter, D. Shen, J. Shao, L. Ding, B.S. White, C. Kandoth, C.A. Miller, B. Niu, M.D. McLellan, N.D. Dees, R. Fulton, K. Elliot, S. Heath, M. Grillot, P. Westervelt, D.C. Link, J.F. DiPersio, E. Mardis, T.J. Ley, R.K. Wilson, T.A. Graubert, Clonal diversity of recurrently mutated genes in myelodysplastic syndromes, Leukemia 27 (2013) 1275–1282.
[80] T.N. Wong, G. Ramsingh, A.L. Young, C.A. Miller, W. Touma, J.S. Welch, T.L. Lamprecht, D. Shen, J. Hundal, R.S. Fulton, S. Heath, J.D. Baty, J.M. Klco, L. Ding, E.R. Mardis, P. Westervelt, J.F. DiPersio, M.J. Walter, T.A. Graubert, T.J. Ley, T.E. Druley, D.C. Link, R.K. Wilson, Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia, Nature 518 (2015) 552–555.
[81] G. Genovese, A.K. Kahler, R.E. Handsaker, J. Lindberg, S.A. Rose, S.F. Bakhoum, K. Chambert, E. Mick, B.M. Neale, M. Fromer, S.M. Purcell, O. Svantesson, M. Landen, M. Hoglund, S. Lehmann, S.B. Gabriel, J.L. Moran, E.S. Lander, P.F. Sullivan, P. Sklar, H. Gronberg, C.M. Hultman, S.A. McCarroll, Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence, N. Engl. J. Med. 371 (2014) 2477–2487.
[82] S. Jaiswal, P. Fontanillas, J. Flannick, A. Manning, P.V. Grauman, B.G. Mar, R.C. Lindsley, C.H. Mermel, N. Burtt, A. Chavez, J.M. Higgins, V. Moltchanov, F.C. Kuo, M.J. Kluk, B. Henderson, L. Kinnunen, H.A. Koistinen, C. Ladenvall, G. Getz, A. Correa, B.F. Banahan, S. Gabriel, S. Kathiresan, H.M. Stringham, M.I. McCarthy, M. Boehnke, J. Tuomilehto, C. Haiman, L. Groop, G. Atzmon, J.G. Wilson, D. Neuberg, D. Altshuler, B.L. Ebert, Age-related clonal hematopoiesis associated with adverse outcomes, N. Engl. J. Med. 371 (2014) 2488–2498.
[83] E. Papaemmanuil, M. Gerstung, L. Malcovati, S. Tauro, G. Gundem, P. Van Loo, C.J. Yoon, P. Ellis, D.C. Wedge, A. Pellagatti, A. Shlien, M.J. Groves, S.A. Forbes, K.

Raine, J. Hinton, L.J. Mudie, S. McLaren, C. Hardy, C. Latimer, M.G. Della Porta, S. O'Meara, I. Ambaglio, A. Galli, A.P. Butler, G. Walldin, J.W. Teague, L. Quek, A. Sternberg, C. Gambacorti-Passerini, N.C. Cross, A.R. Green, J. Boultwood, P. Vyas, E. Hellstrom-Lindberg, D. Bowen, M. Cazzola, M.R. Stratton, P.J. Campbell, C. Chronic Myeloid Disorders Working Group of the International Cancer Genome, Clinical and biological implications of driver mutations in myelodysplastic syndromes, Blood 122 (2013) 3616–3627 (quiz 3699).

[84] L. Melchor, A. Brioli, C.P. Wardell, A. Murison, N.E. Potter, M.F. Kaiser, R.A. Fryer, D.C. Johnson, D.B. Begum, S. Hulkki Wilson, G. Vijayaraghavan, I. Titley, M. Cavo, F.E. Davies, B.A. Walker, G.J. Morgan, Single-cell genetic analysis reveals the composition of initiating clones and phylogenetic patterns of branching and parallel evolution in myeloma, Leukemia 28 (2014) 1705–1715.

[85] O.L. Bohn, K. Hsu, D.M. Hyman, D.S. Pignataro, S. Giralt, J. Teruya-Feldstein, BRAF V600E mutation and clonal evolution in a patient with relapsed refractory myeloma with plasmablastic differentiation, Clin. Lymphoma Myeloma Leuk. 14 (2014) e65–e68.

[86] M.R. Green, A.J. Gentles, R.V. Nair, J.M. Irish, S. Kihira, C.L. Liu, I. Kela, E.S. Hopmans, J.H. Myklebust, H. Ji, S.K. Plevritis, R. Levy, A.A. Alizadeh, Hierarchy in somatic mutations arising during genomic evolution and progression of follicular lymphoma, Blood 121 (2013) 1604–1611.

[87] C. Bodor, V. Grossmann, N. Popov, J. Okosun, C. O'Riain, K. Tan, J. Marzec, S. Araf, J. Wang, A.M. Lee, A. Clear, S. Montoto, J. Matthews, S. Iqbal, H. Rajnai, A. Rosenwald, G. Ott, E. Campo, L.M. Rimsza, E.B. Smeland, W.C. Chan, R.M. Braziel, L.M. Staudt, G. Wright, T.A. Lister, O. Elemento, R. Hills, J.G. Gribben, C. Chelala, A. Matolcsy, A. Kohlmann, T. Haferlach, R.D. Gascoyne, J. Fitzgibbon, EZH2 mutations are frequent and represent an early event in follicular lymphoma, Blood 122 (2013) 3165–3168.

[88] J. Okosun, C. Bodor, J. Wang, S. Araf, C.Y. Yang, C. Pan, S. Boller, D. Cittaro, M. Bozek, S. Iqbal, J. Matthews, D. Wrench, J. Marzec, K. Tawana, N. Popov, C. O'Riain, D. O'Shea, E. Carlotti, A. Davies, C.H. Lawrie, A. Matolcsy, M. Calaminici, A. Norton, R.J. Byers, C. Mein, E. Stupka, T.A. Lister, G. Lenz, S. Montoto, J.G. Gribben, Y. Fan, R. Grosschedl, C. Chelala, J. Fitzgibbon, Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma, Nat. Genet. 46 (2014) 176–181.

[89] H. Bai, Z. Wang, Y. Wang, M. Zhuo, Q. Zhou, J. Duan, L. Yang, M. Wu, T. An, J. Zhao, J. Wang, Detection and clinical significance of intratumoral EGFR mutational heterogeneity in Chinese patients with advanced non-small cell lung cancer, PLoS ONE 8 (2013) e54170.

[90] Z.Y. Chen, W.Z. Zhong, X.C. Zhang, J. Su, X.N. Yang, Z.H. Chen, J.J. Yang, Q. Zhou, H.H. Yan, S.J. An, H.J. Chen, B.Y. Jiang, T.S. Mok, Y.L. Wu, EGFR mutation heterogeneity and the mixed response to EGFR tyrosine kinase inhibitors of lung adenocarcinomas, Oncologist 17 (2012) 978–985.

[91] Y. Yatabe, K. Matsuo, T. Mitsudomi, Heterogeneous distribution of EGFR mutations is extremely rare in lung adenocarcinoma, J. Clin. Oncol. 29 (2011) 2972–2977.

[92] R. Katayama, A.T. Shaw, T.M. Khan, M. Mino-Kenudson, B.J. Solomon, B. Halmos, N.A. Jessop, J.C. Wain, A.T. Yeo, C. Benes, L. Drew, J.C. Saeh, K. Crosby, L.V. Sequist, A.J. Iafrate, J.A. Engelman, Mechanisms of acquired crizotinib resistance in ALK-rearranged lung Cancers, Sci. Transl. Med. 4 (2012) 120ra117.

[93] K.Y. Su, H.Y. Chen, K.C. Li, M.L. Kuo, J.C. Yang, W.K. Chan, B.C. Ho, G.C. Chang, J.Y. Shih, S.L. Yu, P.C. Yang, 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. 30 (2012) 433–440.

[94] P.M. Pollock, U.L. Harper, K.S. Hansen, L.M. Yudt, M. Stark, C.M. Robbins, T.Y. Moses, G. Hostetter, U. Wagner, J. Kakareka, G. Salem, T. Pohida, P. Heenan, P. Duray, O. Kallioniemi, N.K. Hayward, J.M. Trent, P.S. Meltzer, High frequency of BRAF mutations in nevi, Nat. Genet. 33 (2003) 19–20.

[95] J.N. Poynter, J.T. Elder, D.R. Fullen, R.P. Nair, M.S. Soengas, T.M. Johnson, B. Redman, N.E. Thomas, S.B. Gruber, BRAF and NRAS mutations in melanoma and melanocytic nevi, Melanoma Res. 16 (2006) 267–273.

[96] J. Lin, Y. Goto, H. Murata, K. Sakaizawa, A. Uchiyama, T. Saida, M. Takata, Polyclonality of BRAF mutations in primary melanoma and the selection of mutant alleles during progression, Br. J. Cancer 104 (2011) 464–468.

[97] M. Yancovitz, A. Litterman, J. Yoon, E. Ng, R.L. Shapiro, R.S. Berman, A.C. Pavlick, F. Darvishian, P. Christos, M. Mazumdar, I. Osman, D. Polsky, Intra- and inter-tumor heterogeneity of BRAF(V600E) mutations in primary and metastatic melanoma, PLoS ONE 7 (2012) e29336.

[98] R. Nazarian, H. Shi, Q. Wang, X. Kong, R.C. Koya, H. Lee, Z. Chen, M.K. Lee, N. Attar, H. Sazegar, T. Chodon, S.F. Nelson, G. McArthur, J.A. Sosman, A. Ribas, R.S. Lo, Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation, Nature 468 (2010) 973–977.

[99] L.A. Fecher, S.D. Cummings, M.J. Keefe, R.M. Alani, Toward a molecular classification of melanoma, J. Clin. Oncol. 25 (2007) 1606–1620.

[100] E.M. Van Allen, N. Wagle, A. Sucker, D.J. Treacy, C.M. Johannessen, E.M. Goetz, C.S. Place, A. Taylor-Weiner, S. Whittaker, G.V. Kryukov, E. Hodis, M. Rosenberg, A. McKenna, K. Cibulskis, D. Farlow, L. Zimmer, U. Hillen, R. Gutzmer, S.M. Goldinger, S. Ugurel, H.J. Gogas, F. Egberts, C. Berking, U. Trefzer, C. Loquai, B. Weide, J.C. Hassel, S.B. Gabriel, S.L. Carter, G. Getz, L.A. Garraway, D. Schadendorf, Dermatologic Cooperative Oncology Group of Germany. The genetic landscape of clinical resistance to RAF inhibition in metastatic melanoma, Cancer Discov. 4 (2014) 94–109.

[101] H. Shi, W. Hugo, X. Kong, A. Hong, R.C. Koya, G. Moriceau, T. Chodon, R. Guo, D.B. Johnson, K.B. Dahlman, M.C. Kelley, R.F. Kefford, B. Chmielowski, J.A. Glaspy, J.A. Sosman, N. van Baren, G.V. Long, A. Ribas, R.S. Lo, Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy, Cancer Discov. 4 (2014) 80–93.

[102] R. Somasundaram, M. Herlyn, Relapse of melanoma after successful adoptive T-cell therapy: escape through inflammation-induced phenotypic melanoma cell plasticity, Pigment Cell Melanoma Res. 26 (2013) 2–4.

[103] D. Prandi, S.C. Baca, A. Romanel, C.E. Barbieri, J.M. Mosquera, J. Fontugne, H. Beltran, A. Sboner, L.A. Garraway, M.A. Rubin, F. Demichelis, Unraveling the clonal hierarchy of somatic genomic aberrations, Genome Biol. 15 (2014) 439.

[104] S.S. Wang, Y.F. Gu, N. Wolff, K. Stefanius, A. Christie, A. Dey, R.E. Hammer, X.J. Xie, D. Rakheja, I. Pedrosa, T. Carroll, R.M. McKay, P. Kapur, J. Brugarolas, Bap1 is essential for kidney function and cooperates with Vhl in renal tumorigenesis, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 16538–16543.

[105] A. Sankin, A.A. Hakimi, N. Mikkilineni, I. Ostrovnaya, M.T. Silk, Y. Liang, R. Mano, M. Chevinsky, R.J. Motzer, S.B. Solomon, E.H. Cheng, J.C. Durack, J.A. Coleman, P. Russo, J.J. Hsieh, The impact of genetic heterogeneity on biomarker development in kidney cancer assessed by multiregional sampling, Cancer Med. 3 (2014) 1485–1492.

[106] M.H. Voss, A.A. Hakimi, C.G. Pham, A.R. Brannon, Y.B. Chen, L.F. Cunha, O. Akin, H. Liu, S. Takeda, S.N. Scott, N.D. Socci, A. Viale, N. Schultz, C. Sander, V.E. Reuter, P. Russo, E.H. Cheng, R.J. Motzer, M.F. Berger, J.J. Hsieh, Tumor genetic analyses of patients with metastatic renal cell carcinoma and extended benefit from mTOR inhibitor therapy, Clin. Cancer Res. 20 (2014) 1955–1964.

[107] N. Kanu, E. Gronroos, P. Martinez, R.A. Burrell, X. Yi Goh, J. Bartkova, A. Maya-Mendoza, M. Mistrik, A.J. Rowan, H. Patel, A. Rabinowitz, P. East, G. Wilson, C.R. Santos, N. McGranahan, S. Gulati, M. Gerlinger, N.J. Birkbak, T. Joshi, L.B. Alexandrov, M.R. Stratton, T. Powles, N. Matthews, P.A. Bates, A. Stewart, Z. Szallasi, J. Larkin, J. Bartek, C. Swanton, SETD2 loss-of-function promotes renal cancer branched evolution through replication stress and impaired DNA repair, Oncogene (2015)http://dx.doi.org/10.1038/onc.2015.24 (Epub ahead of print).

[108] M. Snuderl, L. Fazlollahi, L.P. Le, M. Nitta, B.H. Zhelyazkova, C.J. Davidson, S. Akhavanfard, D.P. Cahill, K.D. Aldape, R.A. Betensky, D.N. Louis, A.J. Iafrate, Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma, Cancer Cell 20 (2011) 810–817.

[109] N.J. Szerlip, A. Pedraza, D. Chakravarty, M. Azim, J. McGuire, Y. Fang, T. Ozawa, E.C. Holland, J.T. Huse, S. Jhanwar, M.A. Leversha, T. Mikkelsen, C.W. Brennan, 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. 109 (2012) 3041–3046.

[110] J.M. Francis, C.Z. Zhang, C.L. Maire, J. Jung, V.E. Manzo, V.A. Adalsteinsson, H. Homer, S. Haidar, B. Blumenstiel, C.S. Pedamallu, A.H. Ligon, J.C. Love, M. Meyerson, K.L. Ligon, EGFR variant heterogeneity in glioblastoma resolved through single-nucleus sequencing, Cancer Discov. 4 (2014) 956–971.

[111] S. Jones, W.D. Chen, G. Parmigiani, F. Diehl, N. Beerenwinkel, T. Antal, A. Traulsen, M.A. Nowak, C. Siegel, V.E. Velculescu, K.W. Kinzler, B. Vogelstein, J. Willis, S.D. Markowitz, Comparative lesion sequencing provides insights into tumor evolution, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 4283–4288.

[112] A. Brannon, E. Vakiani, B.E. Sylvester, S.N. Scott, G. McDermott, R.H. Shah, K. Kania, A. Viale, D.M. Oschwald, V. Vacic, A.K. Emde, A. Cercek, R. Yaeger, N.E. Kemeny, L.B. Saltz, J. Shia, D.M. Graham, M.R. Weiser, D.B. Solit, M.F. Berger, Comparative sequencing analysis reveals high genomic concordance between matched primary and metastatic colorectal cancer lesions, Genome Biol. 15 (2014) 454.

[113] M.A. Donna, M. Graham, Mahadeo A. Sukhai, Louis Letourneau, Mehran Karimzadeh, Tong Zhang, Mariam Thomas, Michael H.A. Roehrl, Eric Xueyu Chen, Monika K. Krzyzanowska, Malcolm J. Moore, Amanda Giesler, Celeste Yu, Philippe L. Bedard, Suzanne Kamel-Reid, Lillian L. Siu, Yasser Riazalhosseini, Analysis of clonal evolution in colorectal cancer, J. Clin. Oncol. 32 (5 s) (2014).

[114] L.A. Diaz Jr., R.T. Williams, J. Wu, I. Kinde, J.R. Hecht, J. Berlin, B. Allen, I. Bozic, J.G. Reiter, M.A. Nowak, K.W. Kinzler, K.S. Oliner, B. Vogelstein, The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers, Nature 486 (2012) 537–540.

[115] C.D. Greenman, E.D. Pleasance, S. Newman, F. Yang, B. Fu, S. Nik-Zainal, D. Jones, K.W. Lau, N. Carter, P.A. Edwards, P.A. Futreal, M.R. Stratton, P.J. Campbell, Estimation of rearrangement phylogeny for cancer genomes, Genome Res. 22 (2012) 346–361.

[116] N. Cancer Genome Atlas Research, Integrated genomic analyses of ovarian carcinoma, Nature 474 (2011) 609–615.

[117] M. Jamal-Hanjani, A. Hackshaw, Y. Ngai, J. Shaw, C. Dive, S. Quezada, G. Middleton, E. de Bruin, J. Le Quesne, S. Shafi, M. Falzon, S. Horswell, F. Blackhall, I. Khan, S. Janes, M. Nicolson, D. Lawrence, M. Forster, D. Fennell, S.M. Lee, J. Lester, K. Kerr, S. Muller, N. Iles, S. Smith, N. Murugaesu, R. Mitter, M. Salm, A. Stuart, N. Matthews, H. Adams, T. Ahmad, R. Attanoos, J. Bennett, N.J. Birkbak, R. Booton, G. Brady, K. Buchan, A. Capitano, M. Chetty, M. Cobbold, P. Crosbie, H. Davies, A. Denison, M. Djearman, J. Goldman, T. Haswell, L. Joseph, M. Kornaszewska, M. Krebs, G. Langman, M. MacKenzie, J. Millar, B. Morgan, B. Naidu, D. Nonaka, K. Peggs, C. Pritchard, H. Remmen, A. Rowan, R. Shah, E. Smith, Y. Summers, M. Taylor, S. Veeriah, D. Waller, B. Wilcox, M. Wilcox, I. Woolhouse, N. McGranahan, C. Swanton, Tracking genomic cancer evolution for precision medicine: The Lung TRACERx Study, PLoS Biol. 12 (2014) e1001906.

[118] N. Rivlin, R. Brosh, M. Oren, V. Rotter, Mutations in the p53 tumor suppressor gene: important milestones at the various steps of tumorigenesis, Genes Cancer 2 (2011) 466–474.

[119] A. Marusyk, V. Almendro, K. Polyak, Intra-tumour heterogeneity: a looking glass for cancer? Nat. Rev. Cancer 12 (2012) 323–334.