Prioritizing targets for precision cancer medicine

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The implementation of cancer genomic testing into the clinical setting has brought major opportunities. However, as our understanding of cancer initiation, maintenance and progression improves through detailed cancer genomic studies, the challenges associated with driver identification and target classification in the clinical setting become clearer. Here, we review recent insights into cancer genomic testing in the clinical setting, and suggest a target classification approach that considers the levels of evidence supporting the prioritization of tumour drivers for therapeutic targeting in light of complex cancer clonal and sub-clonal structures and clinical successes and failures in the field. We argue that such classification approaches, together with transparent reporting of both positive and negative clinical data and continued research to identify the sub-clonal dynamics of driver events during the disease course, will facilitate inter-trial comparisons, optimize patient informed consent and provide a critically balanced evaluation of genomic testing in clinical practice.

Key words: biomarkers, genomic instability, breast cancer, lung cancer

classical drug development and the promise of tumour profiling

The design and synthesis of molecules to address tumour-specific (somatic) DNA alterations has its early history in drug development for haematologic malignancies. These have been shown to carry specific and pathogenic fusion gene drivers, such as the BCR-ABL fusion of chronic myeloid leukaemia [1] and the PML-RARα fusion of acute promyelocytic leukaemia [2]. In the mid-2000s, three groups demonstrated that patients with metastatic lung adenocarcinoma who responded to a new class of tyrosine kinase inhibitors harboured tumours with mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) gene [3–5]. Thus, primary discoveries of common somatic DNA alterations and other observations of somatic gene-level alterations conferring targeted drug sensitivity have ushered in a new era of drug development that aims to design therapeutic small molecule and antibody-based inhibitors of common, activating mutations in cancer-causing genes. These efforts have been fuelled by the use of next-generation sequencing (NGS) technologies that not only make it straightforward and inexpensive to profile the mutational landscape of tumours genome-wide [6], but also have demonstrated that there are frequently mutated genes that are altered across cancer types that may respond similarly to a specific targeted therapy. The baseline information about frequent genomic alterations in cancer generated in the research setting by sequencing the DNA of thousands of tumours is now being coupled with NGS-based methods that rapidly generate the mutational profile of a cancer genome in the clinical setting to inform genome-guided cancer medicine.

brief overview of tumour profiling techniques

While early targeted therapy companion diagnostics largely consisted of single-gene assays that ascertained the mutation status of frequently mutated genes or ‘hot spot’ mutations, our growing knowledge that (i) many genes are frequently mutated in multiple tumour types and (ii) non-hot spot mutations in the target gene may portend a therapeutic response has increased the number of genes that may require testing in any given patient. Non-small-cell lung adenocarcinoma is a prime example of this paradigm, as described recently [7]. Early examples of hot spot focused multigene diagnostic assays were devised on mass-spectrometry platforms such as the Sequenom MassArray genotyping instrument [8] or on capillary sequencers [9]. More recently, massively parallel, or next-generation DNA sequencing instruments have been implemented for clinical cancer genomics assays that enable broad-based mutational discovery across a scale from several genes, through to sequencing of the whole exome or whole genome. NGS allows detection of mutations and, in some circumstances, copy-number changes. There are now numerous examples of the clinical application of NGS-based methods to predict therapeutic sensitivity to an ever-increasing number of targeted therapies [10, 11].

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Admittedly, the clinical implementation of these methods has not come without significant challenges, many of which will be addressed in this review. While we acknowledge and discuss these challenges, we see a compelling and clinically relevant rationale for the implementation of NGS-based assays that guide therapeutic choices toward the desirable goal of improved outcomes for patients suffering from cancer. Beyond NGS, RNA-seq permits quantifying RNA expression, determining which mutations detected in DNA are being expressed in the tumour cells and detecting fusion transcripts.

**perspective of genomics in the clinical trials setting—where we are now, what the problems are and where we are heading**

Implementation of clinical genomics assessments in the clinical trial setting is now a reality at multiple academic sites. Some key lessons can be drawn from this initial period (summarized in Table 1 and in ref. [14]). First, there is a strong interest and commitment from patients to the potential benefit of precision medicine initiatives typified by enrolment in prospective clinical trials stratified by cancer genomics methods and large molecular screening initiatives. Secondly, high-throughput analysis of tumour material derived from fresh tumour samples or archived blocks is feasible within clinical timelines acceptable for patients with metastatic disease. Thirdly, planned tumour biopsies can be obtained within nationwide programmes with limited risk of complications [19]. However, there is a great variability of tumour cell content according to centres and expertise. Fourthly, this approach allows the identification of actionable targets in up to 64% of patients in a national study carried out in non-small-cell lung cancer [20]. In the same study, a targeted therapy (or trial) matched to the genomic alteration was proposed in 275 of 1007 patients (28%). However, despite the excitement in the field, the matching of an actionable event with a targeted therapy, has led to lower than expected clinical benefit [19]. This review will consider some of the future opportunities and challenges in the cancer genomics clinical arena and propose strategies to prioritize genomic events within an evidence-based framework for drug development (Table 2).

**adapting clinical trial designs to the challenges of cancer heterogeneity**

While the advent of molecular characterization of human cancers has revolutionized clinical trial design and patient selection for these trials, the likelihood of novel agents attaining drug approval based on histopathologic diagnosis alone is diminishing. This is reflected by the fact that five of nine drugs approved by the US Food and Drug Administration in 2013 had associated predictive molecular biomarkers guiding patient selection beyond histology. A new generation of biomarker-driven clinical trials has emerged, recognizing the relevance of evidence from genotyping and the importance of inter-patient heterogeneity in cancer therapeutic responses and outcomes [21].

‘Umbrella’ trials focus on one histology (stem) but evaluate multiple predictive biomarker groups (spokes) to offer target-drug matching under the auspice of one protocol, with notable examples being I-SPY 2 [NCT01042379] and SAFIR-01 in breast cancer [NCT01414933] [19], Master Protocol [NCT02154490] and ALCHIMIST in lung cancer [NCT02194738] [22] and FOCUS4 in colorectal cancer [ISRCTN90061546] [23]. Histology agnostic ‘basket’ trials enrol patients with different tumour types sharing a common molecular aberration into the same basket, these patients are then tested with a specific matching drug. Examples of basket trials include the US National Cancer Institute MATCH study [NCT02154490] and institutional-based basket trials [24]. ‘N-of-one’ trials compare a target-drug matching approach in each individual patient against the most recent, presumably unmatched, regimen on which the patient had experienced disease progression. The ‘WINThER’ trial [NCT01856296] led by the Worldwide Innovative Networks (WIN) Consortium is one of the most illustrative prototypes of this design.

The above-mentioned clinical trials are relevant in oncology in that they underscore the addition of potential therapeutic benefits when molecular profiling information complements histopathologic diagnostic data. However, despite this shifting attention to address inter-patient heterogeneity, limitations remain in the ability of these new clinical trials to tackle dynamic changes in the molecular milieu within individual patients. All of these trials rely upon molecular characterization of tumour tissue from a single location and at a single time point, whether it is obtained via an archived diagnostic specimen or a freshly procured biopsy. Genomic profiling is typically carried out on only one such sample due to reasons such as cost, challenges in tissue access and sufficiency in DNA quality and quantity. Dynamic alterations in clonal and sub-clonal frequencies occur in cancers exacerbated by genomic instability [25], especially when tumour cells are subjected to selection pressures by radiotherapy [26] and systemic treatments [27]. Multiregional evaluations to determine geographical heterogeneity, and molecular characterization of different samples collected over space and time to ascertain clonal evolution [28, 29], are not routinely carried out even in the setting of biomarker-driven clinical trials.

In order to make substantial progress in cancer therapeutics, the ‘next-generation’ clinical trials must take into account intra-tumour heterogeneity within individual patients and utilize a comprehensive classification scheme to guide target prioritization. While the validity of a genomic alteration as an important driver is often determined based on data generated at a population level, the genomic and tumour biology-specific contexts within which an altered gene exists, functions and evolves in a unique individual may not be generalizable. Several large programmatic efforts are ongoing to establish a knowledge base on spatial and temporal heterogeneity at the single-patient level, such as TRACERx in non-small-cell lung cancer [NCT01888601] and BEAUTY in breast cancer [NCT02022202]. Furthermore, rapid autopsy programmes can sample tumour tissue that is otherwise not easily accessible, and enable a retrospective construction of the genomic landscape and interactions with the microenvironment. Another approach that is gaining acceptance for certain tumour types is to profile the diagnostic specimen to establish common driver mutations and then periodically sample the blood or other bodily fluids (urine, e.g.) for direct assay and quantitation of the levels of these drivers. This so-called liquid biopsy approach can circumvent the acknowledged difficulty and
associated morbidity that can accompany subsequent invasive tumour sampling events by using circulating tumour cells or circulating free DNA to monitor therapeutic response or developing resistance.

The next-generation clinical trials can begin at an early time point in a patient’s diagnosis and continue throughout the time course of the cancer (Figure 1). These trials might aspire to incorporate some of the principles in Table 3.

Table 1. Key challenges for precision medicine initiative encompass

1) Implementation in the community
   a) Access to ad hoc screening platforms
   b) Cost implications and affordability
   c) Timelines
   d) Reporting of incidental findings [12]
   e) Education of patient population as to the benefits of screening
   f) Empowering the patient, giving them greater input in treatment decisions and over the risks they are willing to take
   g) Flexible informed consent (see below), while protecting patient privacy [13].

2) Genotype-focused clinical trials
   a) Need to screen thousands of patients [14]
   b) New trial designs with novel statistical challenges [15]
   c) Pre-competitive collaboration between diverse stakeholders (industry, academia, non-profit, regulatory, patient advocate groups etc.)

3) Acceptance by medical community
   a) Incorporating genomics into medical education (to promote test awareness and facilitate interpretation)
   b) Establishing effective ‘tumour boards’ that integrate expertise from diverse disciplines to foster stronger inter-disciplinary collaborations (e.g. between clinicians, pathologists, bioinformaticians etc.)
   c) User-friendly bioinformatics tools and clinical reports that facilitate communication with the patient
   d) Standardization and accreditation by a professional body
   e) Ensuring global access (especially in developing countries).

4) Technical considerations
   i) Tissue preparation (e.g. high tumour purity [16])
   ii) Tissue preservation (e.g. FFPE can induce genomic artefacts [17])
   iii) Choice of sequencing strategy (WGS, exome etc.) and platform (Illumina, Ion Torrent etc.) that will robustly capture the full range of somatic mutation being queried (from SNVs to translocations and copy-number changes).
   iv) Establishing acceptable levels of assay sensitivity and specificity [18].
   v) Development of ‘industry-grade’ bioinformatics tools that are actively maintained by inter-disciplinary teams of developers and bioinformaticians.
   vii) Tools to robustly detect sub-clonal mutations.
   viii) Interpretation of somatic variation data to identify optimally targetable events.
   ix) Development of high-throughput molecular biology techniques for routine functional validation of putative driver events
   x) Establishing evidentiary standards for analytical validity and clinical utility.
   xi) Internationally recognized reporting standards, including efforts to harmonize data formats ([http://genomicsandhealth.org/](http://genomicsandhealth.org/))

5) Trials infrastructure required for third-generation trials is huge
   a) Database development and maintenance to manage diversity of mutational repertoires. Ideally, integration with electronic medical health records.
   b) Expensive long-term lossless raw data storage.
   c) Establishing bio-banks—these allow for case-by-case follow-up validations and investigations.
   d) Temporal and spatial evolution of mutational repertoires

6) Demonstrating the real added value of this approach
   a) Reporting results back to the medical community and the society
   b) Quantifying the true advances
   c) Health economic evaluation of cancer genomic screening

7) Combination strategies to synergize different approaches of personalized medicine
   a) For delaying resistance
   b) For transforming initial improvements in response rates to prolonged progression free and overall survival

prioritization of target identification for clinical trial entry and routine care

The implementation of next-generation sequencing into oncology practice brings opportunities and challenges. The potential for multigene analyses in one assay from one sample brings obvious practical benefits. However, the increased resolution and breadth of sequencing raises challenges of data interpretation,
alignment of bioinformatics algorithms across recruiting clinical trials sites, classification of driver events, turnaround time and reporting timelines of genomics data in the clinical setting. Drug development strategies are rapidly adapting to the use of next-generation sequencing within clinical trial protocols. However, there is minimal consensus regarding the definition of an actionable event and the informatics-defined classification of a driver event required to recruit an individual into a specific clinical study. Lack of consensus in such criteria required for trial entry will make cross-trial comparisons hazardous. Worse still, looser definitions of a driver event resulting in the recruitment of patients into therapeutic studies with passenger mutations of minimal functional significance may result in premature and incorrect conclusions of drug failure.

NGS data provide quantitative information regarding the frequency at which a particular variant is detected in a tumour sample subject to variation in the depth of sequencing coverage, ploidy and tumour purity. As tumour purity increases, so should the frequency of a tumour somatic variant. Ploidy and focal copy-number alterations will also affect the frequency with which a variant is called in the tumour population.

Sub-clonal heterogeneity of a somatic event also influences the frequency with which a variant is called in the cancer cell population. An early driver event (a truncal mutation), present in all or the majority of tumour cells will have a higher variant allele proportion than a later event in tumour evolution that has not been subjected to a selective sweep within the tumour (assuming equivalent tumour purity and copy number), occurring in a subset of cells. Logically, one might expect the targeting of a clonally dominant, truncal mutation to result in optimal tumour control, relative to a sub-clonal mutation present in some cells but not others [30, 31]. However, increasing realization that low prevalence, sub-clonal populations, can (i) support the growth of a dominant population through paracrine signalling [32] and (ii) contribute to the acquisition of drug resistance [33, 34] suggests that targeting sub-clonal driver events may also be required for optimal tumour control.

It is increasingly clear that sub-clones wax and wane through therapy [35] and cancers display dynamic alterations in subclone proportions over both space [28, 29, 36–38] and time [27, 39]. An appreciation of the changing nature of disease both spatially and temporally mandates efforts to adopt serial sampling strategies within clinical care that may include circulating tumour cell or tumour-free DNA analysis to overcome concerns associated with repeat tumour biopsies in the metastatic setting. However, while promising, it must be noted that it has not yet been proven systematically that the diversity of somatic aberrations in the primary and metastatic tumour are fully represented within cfDNA or CTCs.

Incorporating variant allele proportion information into clinical trial design, with appropriate consideration of tumour cellularity and corresponding stromal contamination as well as tumour ploidy, may serve to decipher the impact of tumour heterogeneity upon targeted therapeutic response. The DARWIN

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Table 2. Next-generation clinical trials

<table>
<thead>
<tr>
<th>1) Moving genomics to earlier stages of the disease</th>
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<tbody>
<tr>
<td>a) Molecular screening trials in high-risk population (low-dose CT in smokers coupled with blood circulating biomarker analysis)</td>
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<tr>
<td>b) Use of genomics to guide adjuvant therapies</td>
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<td>c) Use of genomics to improve locoregional disease management</td>
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<th>2) A better understanding of evolutionary trajectories of cancer</th>
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<tr>
<td>a) Longitudinal studies with multiple biopsies and blood sampling points for circulating biomarker analyses (coupled with 2b)</td>
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<tr>
<td>b) Autopsy programmes notably in young cancer populations (below 40), never-smokers</td>
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<td>3) Assessment of bioinformatics tools or algorithms rather than drugs</td>
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Figure 1. Target prioritization and treatment decisions.
clinical trial programme (Deciphering Anti-tumour Response With INTratumour heterogeneity) [NCT02183883] will endeavour to assess relationships between variant allele frequencies and drug response and whether targeting a clonally dominant driver event results in improved progression-free survival intervals compared with targeting the same event present in a subclone, heterogeneous within the cancer cell population.

Of equal importance is how a patient is selected for trial inclusion based on the variant detected and called within a tumour. Since any variant can be either SNP or a true somatic mutation, reference to the patient’s germline DNA is desirable to determine likely somatic mutations. Often, commercial assays do not mandate germline DNA assessment, and comparisons are made with extensive published datasets. Even with this comparison, absent a matched germline control, a rare SNP cannot always be formally excluded. Conversely, the ‘contamination’ of SNP databases with cancer-related somatic mutations could lead to false-negative test results [40].

Even if a true somatic mutation can be firmly established in a candidate oncogene against a germline comparator, establishing its functional role as a driver event in the tumour in question can be problematic [41]. For this reason, non-hot spot mutations or rarer, previously undescribed mutations in oncogenic drivers without functional validation, should be considered with caution before trial inclusion.

Taking the above considerations into account, simply defining the presence or absence of a mutation is unlikely to provide a full picture of the potential evolutionary routes through which the tumour might progress during drug therapy. Likewise, recognizing the relative abundance of competing sub-clones within a tumour that emerge during therapy, might more accurately guide intervention. We argue that within levels of evidence scales for the definition of the presence of an actionable alteration, knowledge of its relative clonal dominance, together with the presence of sub-clonal events promoting acquired resistance, is also likely to be important in defining success or failure of a candidate gene–drug interaction.

Evidence is emerging that establishing clonal dominance of a driver event from one biopsy is not a trivial problem. Small gene panels are particularly limited in this regard. Even with whole exome sequencing, a driver event may appear fully clonal in one biopsy, only to be absent when sampling another area of the tumour, demonstrating the sub-clonal nature of this event [29]. This problem is likely to be exacerbated by differences in tumourcellularity, evoking the need for pathology-based examination of samples when possible, before sequencing. However, knowledge of how stable actionable alterations are, both spatially and longitudinally, through the disease course may begin to distinguish driver events that are often or always clonally dominant from those that are often sub-clonal.

toward a scale to classify actionability of genomic events

As the drug development community embraces the use of NGS for patient stratification, the emergent challenges of data interpretation will require a systematic grading of the levels of evidence associated with each drug target alteration and its association with response to therapy.

The ongoing principle of personalized cancer medicine care in the genomic era consists of sequencing a cancer sample (with or without a matched germline control) in order to identify genomic alterations that could define sensitivity to a specific drug. Therapeutic targeting of a defined driver is aimed at attenuating oncogenic activity to precipitate cancer cell death and eventually tumour shrinkage [42]. One of the current challenges in the field of personalized medicine is to identify oncogenic drivers at the individual level using high-throughput genomics datasets, in order to identify candidate oncogenes that might serve as new drug targets. Defining a driver is not a trivial task since the majority of somatic mutations detected by sequencing methods in a tumour sample are not in bona fide oncogenes or tumour suppressor genes [43], but rather are inconsequential mutations that accumulate over time and are due to DNA replication errors. This problem is exacerbated in hyper-mutant tumours such as melanomas, tobacco-associated non-small-cell and small-cell lung cancers or cancers with microsatellite instability or other DNA repair defects. Methods of driver identification have been optimized to include knowledge of DNA replication timing, gene size and background mutation rate. However, attempts to classify individual driver events in terms of levels of evidence that their therapeutic targeting results in measurable patient benefit, have lagged behind such efforts.
In order to address this need, driver classification systems for clinical implementation have been proposed evaluating whether:

(i) a somatic alteration predicted by bioinformatics algorithms plays a role in cancer progression
(ii) the preclinical data support the somatic alteration as driver of oncogenesis
(iii) the data apply across disease subtypes and body sites
(iv) the somatic alteration defines a subset of patients who are sensitive or resistant to a drug

Based on these four criteria, the genomic alteration is classified according to its likelihood of being a target for therapeutic exploitation. As illustration, Van Allen et al. proposed a 5-level evidence scale to rank prognostic, predictive and diagnostic markers [44].

**problems and pitfalls of driver classifications**

The highest level of evidence to define whether a genomic alteration classifies a driver as a bona fide therapeutic target is derived from prospective clinical trials. Thus, evidence of clinical benefit in a population with the genomic alteration within a well-controlled clinical trial is optimal when classifying a drug target. However, there are at least five possible pitfalls that need to be considered within driver classification systems.

First, there must be some evidence to demonstrate that patients who do not harbour the genomic target are unlikely to respond to therapy. As illustration, trastuzumab has been shown to be effective in ERBB2 amplified, but not ERBB2-normal metastatic breast cancers [45]. Nevertheless, especially when preclinical data are robust, it is not always possible to generate efficacy data in a biomarker negative cohort.

Secondly, the data must have been generated in the disease for which the therapy is considered. Although basket trials that include genomic alterations across tumour types are popular, evidence is emerging that the same genomic alteration may have distinct epistatic interactions across different tumour types and, therefore, the biological consequences of attenuating the genomic target may differ between tumour types. As illustration, B-RAF V600E mutations define sensitivity to vemurafenib in melanoma or to dabrafenib in NSCLC [46]. In contrast, the predictive value of BRAF V600E in colorectal cancer may be diminished due to feedback activation of EGFR [47]. Similarly, ERBB2 amplification, although a validated target in breast cancer, was not associated with sensitivity to ERBB2 inhibitors in endometrial cancers presenting with the same genomic alteration [48]. These data emphasize the need to classify targets validated in the disease under investigation and not assume that the identical genomic event as a cancer driver were available. As clinical trial NGS approaches broaden their genomic scope on an individual patient basis the discovery of such aberrations of uncertain significance will become more frequent.

International efforts are ongoing to develop a catalogue of cancer-related genes. Lawrence et al. reported a list of 291 cancer-related genes that are either shared between tumour types or specific to some cancers and are termed high confidence drivers [52]. Interestingly, they concluded their study by the observation that, with the exception of early breast cancer, there is still scope to discover new cancer-related genes in specific cancers [43]. Some caution about calling genes ‘cancer-related’ should be exercised since not all mutations have an equivalent impact on the function of the resulting protein. Ultimately, the definition lies in precise biological investigation of each variant in the appropriate biological system. When one considers the possibility of subclonal driver events, beyond the limits of current sequencing and informatics detection methods, that might foster the expansion of individual tumour clades, the number of driver events operating within individual tumours may expand further.

Based on all these considerations, we have developed a scale to classify genomic alterations according to their likelihood of being therapeutic targets. This scale is reported in Table 4 and summarizes many of the discussion points reported above. Conventional clinical end points including objective response and PFS will be used to define these levels of evidence. One could argue that more modern tools for treatment evaluation are needed. As illustration, only tumour shrinkage at the site of biopsy could be taken into account. Nevertheless, these new
methods currently lack harmonization and clinical relevance to be included in treatment decision tools. Levels of evidence associated with each target will be associated with a footnote stating the properties of the drugs supporting the evidence. Several scenarios could be met. First, the drug could directly hit the target. In this specific case, it will be mentioned whether the drugs are specific to the target or have known off-target effects, based on IC50. Secondly, the drugs supporting the evidence could hit the pathway but not the target. Finally, in some cases, the target will be relevant only when hit concomitant with other targets. Since some drugs are initially reported to hit a target, while they actually do no’t (e.g. iniparib), the level of evidence should take into account whether responses are observed with a single drug or with several drugs from the same family. Integrating knowledge about germline mutations such as BRCA1, BRCA2, PTEN will be necessary since these alterations could be associated with drug sensitivity. This level of evidence scale has two aims. First, it will allow the stratification of clinical trials testing personalized medicine approaches according to the likelihood that a defined alteration is a bona fide target,
facilitating comparisons between clinical studies and hopefully clarifying the likelihood of response. This will also enable optimal interpretation of results reported by clinical trials testing the clinical utility of genomic assays to identify therapeutic targets. Secondly, it should optimize the manner in which patients are informed about the risks and potential benefits of such a study and the reporting of more transparent and accurate information to the patients who have been consented to genetic testing. Indeed, current genomic reports only provide information about actionability and availability of targeted therapies. Reporting genomic results according to a classification scale should improve the interpretation of the implications of the results by both the oncologist and the patient.

**defining characteristics of drivers within a tumour**

Moving beyond the level of evidence scale based on clinical trials reported in Table 4, new ways of classifying a potential target need to be devised by the community based on the characteristics of the genomic alteration. Such systems will have to address whether the gene in question defines a known oncogenic driver, the characteristics of the somatic mutation and whether it has been reported before (e.g. previously reported on COSMIC), functional evidence in silico, in vitro or in vivo that the genomic alteration activates protein or has impact on drug sensitivity [41]. In addition, evaluation of whether the alteration is clonally dominant or sub-clonal within a tumour, with clonally dominant drivers prioritized for drug development approaches may become increasingly important.

**conclusions: toward the development and implementation of a level of evidence scale for target prioritization**

Figure 2 summarizes the current manuscript and reports a possible decision tree approach to identify targets in each individual. In order to achieve this goal, levels of evidence for target prioritization together with attempts by the community to classify potential therapeutic targets will become increasingly important as cancer genomic sequencing becomes more widespread and therapeutic options expand. As our knowledge of cancer driver events improves and our ability to distinguish sub-clonal from clonal drivers between and within cancer subtypes is more refined and informed, driver classification systems may have to consider heterogeneity of driver events within individual tumours for optimal therapeutic targeting. Regular consensus meetings and transparency of data reporting (both positive and negative) will likely form a vital component of research community efforts to share experience and inform the next generation of clinical trials. As reported before, this manuscript emphasizes the need to develop level of evidence for each target. The following lines describe some issues related to this challenge. Data capture and interpretation will be important steps when defining level of evidence for a target. Data could be captured from literature review, public database and individual institutions or companies. One major issue will be to capture negative studies, since these latter ones are less likely to be published. These negative data will be retrieved either from public registry of negative studies (www.winregistry.com) or directly from clinical investigators through clinicaltrials.gov. The data capture and analyses will be done by dedicated experts in methodology, discussed during workshops and other scientific meetings, and an updated classification system will be released frequently. All these efforts should optimally be done under the sponsorship of an international scientific society. The vision would be to add the level of evidence associated with each alteration in genomics reports.

Finally, although target prioritization is a major stake, it must be emphasized that drug discovery, development and testing will certainly be the major bottlenecks of precision medicine. Indeed, drugs are missing for lot of major drivers, including KRAS, and rapid drug development is needed to increase the panel of targets for which drugs are available.

**disclosure**

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**references**


