

Cancer evolution: the final frontier of precision medicine?

Over the last 2 years, there have been an unprecedented number of publications focused on cancer evolutionary processes in solid and haematological cancers, a trend that is set to continue over the next decade. In this editorial, insights and future perspectives of these studies as well as the research priorities for the *Annals of Oncology* Precision Medicine editorial board will be discussed.

It is increasingly clear that many advanced tumours follow a branched, Darwinian evolutionary trajectory. This has been demonstrated in childhood ALL [1], pancreatic cancer [2, 3], colorectal cancer [4], clear cell renal carcinoma [5, 6], breast cancer [7, 8] and prostate cancer [9] among others. Next-generation sequencing studies have demonstrated that cancers share common clonal origins marked by early founder mutations and/or DNA copy-number events. Sub-clones are defined by mutations that occur later in cancer evolution, occurring in some cells but not others. Following branched evolution, multiple sub-clones can co-exist, spatially separated within the same tumour or intermixed within the same biopsy. Importantly, the presence of sub-clones and the resulting intra-tumour heterogeneity is not synonymous with branched evolution; linear evolution with incomplete selective sweeps may still result in sub-clonal intermixing and intra-tumour heterogeneity [10].

We have also learned that there is order within seemingly chaotic heterogeneous tumour genomes. That is parallel evolution of cancer sub-clones is witnessed in tumours where distinct somatic aberrations converge on the same gene in separate sub-clones within the same tumour [5, 6].

There is also unexpected heterogeneity in recurrent DNA copy-number events, that is losses or gains of whole or parts of chromosomes [11]. These events are recurrently witnessed in individual tumour types and are considered drivers of disease biology in their own right.

How can we leverage the results of these lessons to improve clinical trial design and inform future drug discovery approaches?

defining the clinical impact of intra-tumour heterogeneity

First, there is an urgent need to define the impact of intra-tumour heterogeneity on drug response, biomarker validation and clinical outcome in prospective clinical trials. What is the impact of intra-tumour heterogeneity on disease outcome and how do cancer selection pressures, both micro-environment and

treatment related, modulate cancer evolutionary trajectories? We need to understand whether targeting a clonally dominant (trunk) driver results in improved progression-free survival outcomes compared with targeting the same driver when it is sub-clonal, present in the tumour branches, detectable in some sub-clones but not others. We also need to determine high risk sub clones harbouring driver events that might themselves be targetable to limit tumour progression.

Increasing evidence in NSCLC and other solid tumours suggest that the selection of resistant sub-clones during the disease course is responsible for the acquisition of drug resistance and therapeutic failure [12–15]. Intra-tumour heterogeneity and cancer sub-clonal diversity may contribute to the high failure rate of oncology drugs relative to other medical specialties where drugs are applied to stable somatic genomes rather than unstable genomes found in cancers.

Secondly, there is a need to both define and understand the relevance of sub-clonal somatic events that confer resistance to therapy in tumours before the initiation of therapeutics targeting trunk drivers. Evidence in NSCLC suggests that the presence of a low-frequency gatekeeper mutation, T790M, in adenocarcinomas with EGFR-activating mutations before the initiation of EGFR tyrosine kinase inhibitor therapy, is associated with poorer progression-free survival following EGFR tyrosine kinase inhibitor therapy [13]. In addition, it is increasingly apparent that a single-drug resistance somatic event might not be solely responsible for treatment failure. Studies in EML4-ALK-driven NSCLCs have demonstrated at least two distinct sub-clonal ALK mutations conferring resistance to Crizotinib in the same patient [15] as well as evidence for multiple distinct resistance mechanisms to Crizotinib in the same patient [14]. It seems logical that the same rules will apply to targeted therapies in general and that intra-tumour heterogeneity, and the bewildering capacity for the generation of drug-resistant sub-clones that ensues, cannot be ignored.

Intra-tumour heterogeneity, presenting as the spatial separation and temporal dynamics of sub-clones requires consideration of tumour sampling bias in the pursuit of biomarker validation strategies. How can a tumour's genomic landscape be effectively defined through the analysis of one snapshot biopsy at one point in time?

Over the next few years, we will gain deeper insight into the relationships of primary tumours with their metastatic sites as well as phylogenetic relationships between metastatic sites. Important questions will be addressed such as how diverse is the driver landscape between sites of metastatic disease? How many driver events are operating in a single patient when all sites of metastatic disease are considered, and what will be the impact of these data on future drug development strategies?

exploiting parallel evolution converging on single genes or pathways

The finding of order within seemingly chaotic cancer genomes suggests that there are severe constraints to cancer evolution that might be therapeutically exploitable. Evidence that the same gene or signal transduction pathway is recurrently affected within the same tumour through different somatic events provides clear evidence of the finite genetic routes through which some tumours can progress [5, 6]. If we understood more about the host germline, the tumour micro-environment and early founder events within individual tumours, could we predict the next evolutionary move of the tumour? Future research in larger cancer cohorts will undoubtedly reveal pages of the rule book by which cancers play, which might lead to new therapeutic approaches to forestall the next evolutionary move. It is likely that epistatic genetic relationships force sub-clonal populations down distinct evolutionary routes. Deciphering, and in due course exploiting, these dependencies, with the aim of forcing tumours through lower risk evolutionary genetic routes may finesse this complex clinical problem for patient benefit.

tumour macro-evolution

Thirdly, for the most part, precision cancer medicine is focused on the identification, biological evaluation and subsequent targeting of oncogenic mutational drivers of disease biology, rather than identifying approaches to target the products of recurrent DNA copy-number driver events that may alter gene dosage of hundreds of genes in an individual tumour. Structural and numerical alterations in chromosomes that commonly occur in solid tumours can be considered a macro-evolutionary event, altering the copy number and expression of many genes at a time, as opposed to single-point mutations in individual genes that occur at a micro-evolutionary level. Increasing evidence that DNA copy-number changes can be just as heterogeneous within a tumour as somatic point mutations [11], suggests that further work is required to decipher the early copy-number alterations in tumour evolution, ubiquitous throughout the tumour.

Deciphering which genes encoded within regions of recurrent copy number gain and loss contribute to disease biology and developing clinical approaches to target them is a key area for development. Recent data from the SAFIR01 trial demonstrate the logistical, bioinformatics and clinical challenges required to initiate such screening approaches to define recurrent DNA copy-number changes and subsequently target them at a national level.

keeping pace with cancer evolution

So, how can drug development strategies keep pace with such bewildering tumour sub-clonal dynamics? It seems likely that methods of single-cell circulating tumour cell and pooled circulating free DNA genomic analysis may contribute to resolving spatial and temporal dynamics of tumour evolution [16]. Improvements in data processing and bioinformatics will be required to fully integrate developments in cancer genome sequencing into the clinical

Table 1. Research priorities for precision medicine

- a. Detailed patient studies deciphering mechanisms of drug resistance (e.g. Elucidation of pre-existing clones or sub clones conferring resistance or acquisition of somatic events conferring resistance through the disease course)
- b. Methods to quantify intratumour heterogeneity
- c. Definition and elucidation of driver events in the presence of sub clonal heterogeneity
- d. Insights into tumour evolution (e.g. molecular case reports illustrating novel routes or mechanisms of tumour evolution)
- e. Integration of other approaches within a precision medicine framework (immunology, host- microenvironment tumour interactions, circulating tumour biomarker methodologies)
- f. Implementation of new technologies for precision medicine
- g. Novel bioinformatics approaches to decipher cancer evolution and tools for precision medicine
- h. Biomarker validation/clinical qualification

trial setting in order to decipher cancer sub-clonal dynamics in real time.

Tumour genetic heterogeneity might provide a tumour's vulnerability through the presentation of diverse tumour neo-antigens to the immune system. The immune system seems uniquely placed to manage such a task therapeutically. Recent data demonstrating improved activity of immunotherapy approaches in smoking-related NSCLCs, a tumour type with a high mutational load, are encouraging [17].

Intra-tumour heterogeneity and cancer evolution might be considered the final frontier of cancer medicine. The Precision Medicine editorial board for *Annals of Oncology* will prioritize publications in these key areas (Table 1). Clinical management of the evolving tumour genomic landscape will need closer scrutiny in order to turn precision medicine from a blunt to a razor-sharp tool necessary to achieve improvements in patient outcome.

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