Editorial

Understanding Intra-tumour Heterogeneity—The Next Holy Grail of Cancer Therapeutics?

Joline SJ Lim, [1]*MBBS, MRCP (UK), M Med (Int Med)*, Soo Chin Lee, [1]*MBBS, MRCP (UK), MMed (Int Med)*

Advances in strategies to characterise tumours in the last decade have uncovered distinct molecular subtypes with prognostic and predictive relevance in both solid tumours and haematological malignancies. While conventional chemotherapy remains the mainstay in cancer therapeutics, the recognition of distinct "actionable genotypes" has led to a new paradigm shift towards targeted therapy, both as primary or adjunct treatment with chemotherapy, to improve patient outcomes.

Inter- and Intra-tumour Heterogeneity

Inter-tumour heterogeneity (between patients) is a long recognised phenomenon, but more recently, there is revelation of a surprisingly high degree of tumour heterogeneity even within the same patient (intra-tumour heterogeneity), with marked diversity demonstrated both between different geographical locations of the same tumour (spatial heterogeneity) and between primary and metastatic tumour sites (temporal heterogeneity).[1] PET-CT imaging with radionucleotide-tagged estradiol have shown varying estrogen receptor distribution between primary and metastatic sites of breast cancer, highlighting the pitfall of relying on a single tumour biopsy to guide treatment choices. These observations also account for variable treatment response in different tumour sites often observed in the same patient.[2] Interestingly, while one expects metastatic lesions and primary tumours to share common ancestral lineage, whole-exome sequencing of tissue samples of brain metastases with matched primary tumour and normal tissue identified subpopulations in both primary and metastatic lesions that bear little resemblance to the common ancestor. This suggests continued, independent evolution of subclones in distinct sites within the same patient, possibly accounting for temporal and spatial tumour heterogeneity.[3] Much of this heterogeneity exists even before treatment, but the exertion of selection pressure with anti-cancer therapy further encourages proliferation and evolution of tumour subpopulations that are resistant to initial therapy.

Clonal Evolution and Darwinian Selection

While anti-cancer therapy, in particular, targeted therapy often leads to dramatic responses in the initial stages, this "honeymoon period" is usually short-lived, with inevitable development of resistance and disease progression—but why so? Similar to the Darwinian theory, the clonal evolution model of tumour carcinogenesis first proposed by Nowell in 1976[4] hypothesised that external factors like environment and therapy leads to selection pressure for clonal populations that are resistant to proliferate while susceptible populations perish. This bottle neck effect may manifest as a period of partial response to therapy, but quickly results in tumour progression once there is clonal outgrowth of resistant populations.[5] In addition to resistant subpopulations present in tumours prior to systemic chemotherapy, the intrinsic genomic instability of tumour cells also leads to accelerated adaptive mechanisms that allow susceptible populations to develop new mutations, thus conferring acquired resistance. This is well documented in lung cancer patients with epidermal growth factor receptor (EGFR) mutations who are initially sensitive to EGFR tyrosine kinase inhibitors, but subsequently develop resistance through acquisition of a secondary mutation T790M.[6] Further complicating this is the observation that secondary mutations can be highly heterogeneous, with the possibility of simultaneous development of 2 or more such mutations, as has been described in lung cancer patients with ALK translocation who develop resistance towards ALK inhibitor crizotinib.[7] This drive of tumour cells to thrive despite anti-cancer therapy is an exhibition of "survival of the fittest" at its best.

Repeated Tumour Sampling to Characterise the Constantly Evolving Tumour

The presence of tumour heterogeneity poses great challenges to cancer therapeutics. What does this entail towards our pursuit of cancer eradication, and are we always going to be blindsided by its chameleon-like nature or can we develop a counter-strategy? With the understanding of

[1]Department of Haematology-Oncology, National University Cancer Institute, National University Health System, Singapore
Address for Correspondence: Dr Soo Chin Lee, Department of Haematology-Oncology, National University Cancer Institute, National University Health System, 1E Kent Ridge Road, NUHS Tower block, level 7, Singapore 119228.
Email: soo_chin_lee@nuhs.edu.sg

constant change in clonal composition of tumour cells, it is prudent to revisit our usual practice of relying on a single tissue biopsy at diagnosis to determine all future courses of therapy. The fluidity in genetic composition suggests the need for constant surveillance of new sub-clones, and multiple biopsies during different time points at various tumour sites may be required for proper characterisation to aid rational drug choices. However, this is admittedly difficult in the clinical setting both in terms of accessibility to tumour sites and acceptability to patients. To overcome this problem, development of reliable, non-invasive techniques to monitor tumour burden becomes increasingly urgent. Circulating tumour cells (CTC) and DNA (ctDNA) have shown promise, with recent studies concluding that levels of ctDNA with targeted sequencing of crucial genes, such as *PIK3CA* and *TP53*, correlated with treatment response in breast cancer.[8] Further studies are ongoing to investigate similar non-invasive methods to monitor emerging resistance patterns.

Therapeutic Implications of Intra-tumoural Heterogeneity and Clonal Evolution

The success of molecular therapy relies heavily on the presence of an oncogenic addiction pathway that when targeted, causes downstream cell death and thus tumour eradication. While tumour heterogeneity may seemingly be the nemesis of targeted therapy, increasing understanding of "branched evolution" of clonal populations may identify "trunk mutations" occurring early in pathogenesis that can be targeted simultaneously with a combination of drugs to prevent low frequency subpopulations from becoming resistant dominant populations later in the disease.[9] Newer technologies like next generation sequencing that offer high sensitivity testing to detect minor subpopulation of cells could be crucial to rationalise such therapeutics.

Another approach towards eradication of residual disease is to consider surgical resection post-chemotherapy. This has been tested in advanced gastro-intestinal stromal tumours where patients with residual disease, believed to be subpopulations that are resistant to chemotherapy, underwent surgical resection. Patients with stable disease or limited progression on initial therapy had prolonged overall survival after surgical resection but limited advantage was gained in patients with generalised progression.[10] This approach remains controversial but could warrant evaluation in selected patients.

The landscape of tumour heterogeneity is rapidly evolving and fast gaining recognition as an important issue that needs to be addressed in today's climate of targeted therapy. While its impact on treatment response and resistance is still not well documented, it is possible that with the advent of newer genomic technologies and more intensive study on the longitudinal impact of targeted therapy in trials, we may still be able to overcome these limitations and gain trajectory towards personalised medicine. The first step will be to acknowledge and embrace that change is the only constant.

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