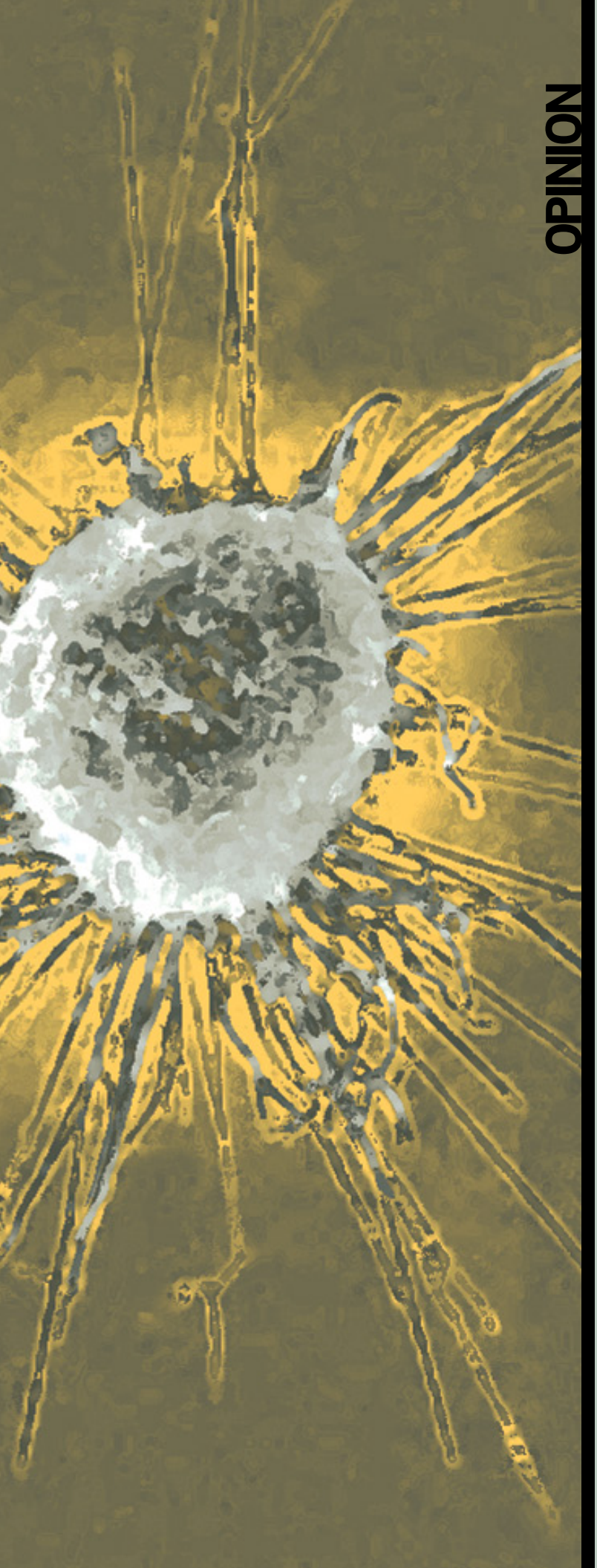

Tumour heterogeneity and treatment:

ONE STEP FORWARD OR TWO STEPS BACK?

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The pillars to establish cancer as a genetic disease began to be built in 1890 with David von Hansemann's description of mitosis in 13 different carcinoma samples.[1] Observing under a microscope, he found anomalies such as multipolar mitoses and asymmetric distribution of chromosomes, and postulated that aberrant cell divisions were responsible for the change in chromatin content found in cancer cells. After the turn of the century, in 1914, Theodor Boveri detailed these cytogenetic anomalies, suggesting that an incorrect combination of chromosomes generates a proliferative malignant cell, which is then capable of passing these functional defects to its daughter cells.[2] The foundation of cancer as a genetic disease was laid.

The pathophysiology of cancer was explored using light microscopy in the ensuing decades, leading to the identification of morphological—i.e., histopathological—differences within and between tumours. Such intratumour and intertumour heterogeneity was linked to disease prognosis and risk-stratification for therapeutic interventions. For example, in medulloblastoma (MB)—the most frequent malignant paediatric brain tumour—morphological differences among tumour cells separate this childhood brain tumour into five histological subtypes: classical, desmoplastic/nodular, MB with extensive nodularity, anaplastic MB, and large-cell MB.[3] Patients with anaplastic and large-cell MB tend to be stratified into the high-risk subgroup, whose treatment consists of higher doses of radiation and longer cycles of chemotherapy.[4] Therefore, heterogeneity as determined by histology was used to guide treatment prior to the advent of genomic high-throughput sequencing in the late twentieth century.

Unfortunately, data collected over the past 50 years for various cancers, including MB, indicate inconsistencies in clinical outcomes based on histopathological subtypes.[4] Although the cellular architecture of a tumour when viewed under a microscope may provide clues into *regional* changes in tumour cell phenotype, one may not appreciate the genomic *landscape* of the tumour. Robert Weinberg's discovery in 1982 of the first oncogene,[5-6] *Ras*, in bladder carcinoma cell lines, shifted the therapeutic framework for cancer from histology-based to one guided by identifying genomic anomalies. Within the last decade especially, several solid tumour malignancies (breast,[7] colon,[8] and brain[4,9]) have benefitted from molecular techniques that analyze the DNA fingerprints of a tumour—e.g., high-throughput gene expression microarray, DNA copy number, and transcriptome analyses. These genomic platforms have discovered countless somatic mutations, copy number alterations, and cytogenetic anomalies that cluster tissue-specific tumours into various molecular subgroups.[4,7-9] MB, again, serves as a prime example. Its recent classification based on genomic differences re-conceptualized the heterogeneity that exists within the five histopathological subtypes,[4] while contextualizing the role of key developmental cell signalling pathways. Specifically, the current consensus for the molecular classification of this childhood brain tumour consists of four subgroups, each distinct in terms of prognosis and predicted therapeutic response.[4] Groups 1 and 2 are characterized by upregulation of genes in the Wnt and Sonic hedgehog (Shh) pathways, respectively. These two subgroups are separated from each other and other subgroups using bioinformatics and both are associated with improved clinical outcomes. Groups 3 and 4, in comparison, are characterized by a greater propensity for metastatic disease and poor clinical outcomes. There has not been a signalling-pathway phenotype attributed to Groups 3 and 4 and, therefore, they remain poorly understood.

Although tumours may arise from different tissues—e.g., breast, pancreas, or cerebellum (in the case of MB)—they are, in fact, unique diseases as indicated by their molecular subgroups. These findings suggest the need for personalized therapy targeted against the particular anomalies present in a given subgroup.

The identification of unique molecular subgroups speaks to the wide range of intertumour heterogeneity in cancer. Although tumours may arise from different tissues—e.g., breast, pancreas, or cerebellum (in the case of MB)—they are, in fact, unique diseases as indicated by their molecular subgroups. These findings suggest the need for personalized therapy targeted against the particular anomalies present in a given subgroup. The clinical utility of this hypothesis is being assessed through preliminary trials exploring the use of Shh pathway inhibitors in patients with Shh-driven Group 2 MBs.[10] This drive for *personalized medicine* in cancer treatment encourages further segregation of subgroups into subtypes, as is the case for Group 3 MB: high-risk patients are stratified into those with amplification of the *MYC* gene (Group 3α), and those without it (Group 3β).[4] To the general public, the age of molecular-subtype-guided therapy brings hope of a new world in which patients will undergo a needle biopsy of a tumour in an outpatient clinic, followed by a rapid turn-around in which an active treatment will be devised on the basis of the distinct genetic characteristics of their tumour. However, serious flaws exist in this portrayal of oncology treatment: this depiction considers only *inter*tumour heterogeneity—the molecular differences between tumours—and does not incorporate recent oncological research suggesting an underestimation of the heterogeneity within a tumour, *intra*tumour heterogeneity.

A critical appraisal of the current practice of molecular subtyping to characterize intertumour heterogeneity to personalize anticancer treatments bears two major limitations. First, the molecular profile used to describe an entire tumour is based upon a single, small specimen of the bulk tumour. This assumes the genomic landscape of the tumour is consistent throughout the entire mass, a concept that is challenged by recent investigation.[11] Second, genetic and cytogenetic anomalies identified by subtyping have yet to be investigated in terms of their functional significance, that is, whether such mutations significantly alter—whether reducing or enhancing—protein function.[11] These limitations are compounded by our rudimentary knowledge of the extent of intratumour heterogeneity at the genetic and epigenetic levels, since current gene expression platforms cannot resolve differences in mRNA expression or DNA copy number alterations between individual cells of the same tumour.

Charles Swanton's group from Cancer Research UK's London Research Institute recently shed light on intratumour heterogeneity using deep-sequencing technologies, which assess genetic differences at the single-nucleotide level.[12] They examined multiple tumour biopsy samples from four consecutive patients with metastatic renal carcinoma before and after cytoreductive nephrectomy, a surgical treatment for kidney cancer patients. The results were quite astounding. Multiregional genetic analysis of the four tumours showed intratumour heterogeneity in every tumour: 65% of somatic mutations found in single biopsies were not uniformly detectable throughout all sampled regions within the *same* tumour. The most concerning finding was their detection of gene expression signatures that indicated both good and poor prognosis in different regions of the *same* tumour. These findings have major implications for oncology treatment, which is currently moving toward therapy driven by various gene expression platforms that may not account for this intratumour heterogeneity. With respect to MB, these results suggest that a tumour may appear to have a Group 2 Shh-driven MB profile in one region based on transcriptome

analysis, but may be a Group 3 or Group 4 MB in other regions of the tumour. Therefore, decisions based upon a single tumour biopsy, the standard for tumour diagnosis and the cornerstone of personalized medicine, cannot be considered representative of the landscape of genomic abnormalities in a tumour.

The question remains: does personalized medicine have a role in oncology? Interestingly, the work done by Charles Swanton's group confirms the presence of genetic lesions expressed in the original tumour cells that are consistent across the majority of cells within the bulk tumour.[12] Therefore, if one was to target these so-called *actionable* mutations,[11] thought to be early drivers of disease leading to the ubiquitous somatic events present in every tumour subclone and region, one may effectively target the top of the tumour's genomic hierarchy. However, the difficulty remains in discerning such *actionable* mutations from other mutations that have evolved over the course of tumourigenesis, responding to cues received within the tumour milieu. One likely and possible approach is to combine the breakthroughs made in cancer genomics and stem cell biology. The cancer stem cell (CSC) hypothesis suggests that a relatively small fraction of tumour cells, termed CSCs, initiate and maintain tumour growth.[13-15] They contrast with other cells of the bulk tumour, which are characterized by a limited proliferative capacity and a more specified lineage potential. A CSC maintains two key properties: self-renewal and differentiation.[13-15] Self-renewal is defined as the ability of a parental cell to generate an identical daughter cell, and a second cell of the same or different phenotype. Through the process of differentiation, a CSC gives rise to the heterogeneous cell lineages that comprise the tumour. Consequently, by isolating tissue-specific CSCs through unique cell-surface proteins, one may comparatively profile the genetic landscape of the CSC with those cells of the bulk tumour mass, thereby identifying *actionable* mutations at the top of the genomic landscape, in cells (CSCs) at the top of the tumour hierarchy.

The identification of intratumour heterogeneity may appear to have brought cancer treatment two steps back. But the future outlook for patients might now include more efficacious, novel therapeutic targets at cell-specific *actionable* mutations, potentially moving the field of oncology, in terms of understanding tumour biology and personalized medicine, one giant leap forward.

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