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Review

The translational challenges of precision oncology

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SUMMARY

The translational challenges in the field of precision oncology are in part related to the biological complexity and diversity of this disease. Technological advances in genomics have facilitated large sequencing efforts and discoveries that have further supported this notion. In this review, we reflect on the impact of these discoveries on our understanding of several concepts: cancer initiation, cancer prevention, early detection, adjuvant therapy and minimal residual disease monitoring, cancer drug resistance, and cancer evolution in metastasis. We discuss key areas of focus for improving cancer outcomes, from biological insights to clinical application, and suggest where the development of these technologies will lead us. Finally, we discuss practical challenges to the wider adoption of molecular profiling in the clinic and the need for robust translational infrastructure.

INTRODUCTION

The global burden of cancer is increasing. In 2020, there were an estimated 19.3 million new cancer cases worldwide, with almost 10 million deaths (Sung et al., 2021). The incidence of cancer cases is expected to rise by 47% to 28.4 million by 2040, with widening inequalities between countries, ethnicities, and socioeconomic status. The reasons for the increase in incidence include both a growing and aging population. However, for multiple tumor types, the age-specific risk is also increasing (Smittenaar et al., 2016). Environmental exposures are linked to the increasing age-specific risk of many tumor types, in part driven by the consequences or drivers of climate change through increased exposure to environmental carcinogens, such as air pollution and UV exposure. Moreover, extreme weather patterns and rising sea levels are likely to drive population displacement, further exacerbating socio-economic and international disparities in cancer outcomes (Nogueira et al., 2020).

Precision oncology refers to the concept of cancer treatment strategies that are based on the distinct molecular characteristics of a tumor. Although these characteristics are historically defined by genetic mutations, defining these patterns to establish treatment strategies has proven more complex due to key considerations, such as the transcriptome, proteome, and tumor microenvironment, in governing tumor development and treatment response. The advent of high-throughput genomic technologies has brought with it exciting potential to further unravel early- and late-stage disease biology. In this review, we reflect on the impact that some of the discoveries in genomics have made on our understanding of cancer initiation, cancer prevention, early detection, adjuvant therapy and minimal residual disease monitoring, cancer drug resistance, and cancer evolution from early- to late-stage disease. We discuss a number of key areas of focus for improving cancer outcomes, from biological insights to clinical application, and suggest where the development of these technologies will lead us. Finally, we suggest knowledge gaps that require complementary approaches to fully address.

CANCER INITIATION

Cancer initiation describes the process of molecular events that lead a normal cell to transform into a cancer cell. In this section, we discuss how the discoveries that have supported this view have led to the conception of precision oncology and look at a number of key research areas in the field of cancer initiation (Figure 1).

Genomics and cancer genes

The concept of cancer as a genetic disease has been considered for over 100 years. This has been underpinned by a number of key events, such as the heritability of breast cancer reported by Pierre Paul Broca (Broca, 1866), the observation of aberrant mitoses by David Von Hansemann (Hansemann, 1890), and the hypothesis of Theodor Boveri (Boveri, 1914), that abnormal chromosomal segregation was sufficient to cause malignant proliferation. The discovery that viral transmissibility of a chicken sarcoma through injection of cell-free infiltrates by Peyton Rous in 1910 laid the foundations for the first discovery of a cancer-related gene, *SRC*, in 1976 (Martin, 2004; Stehelin et al., 1976).

By the year 2000, around 300 cancer-related genes had been identified (Futreal et al., 2004; Martínez-Jiménez et al., 2020). The advent and widespread application of next-generation

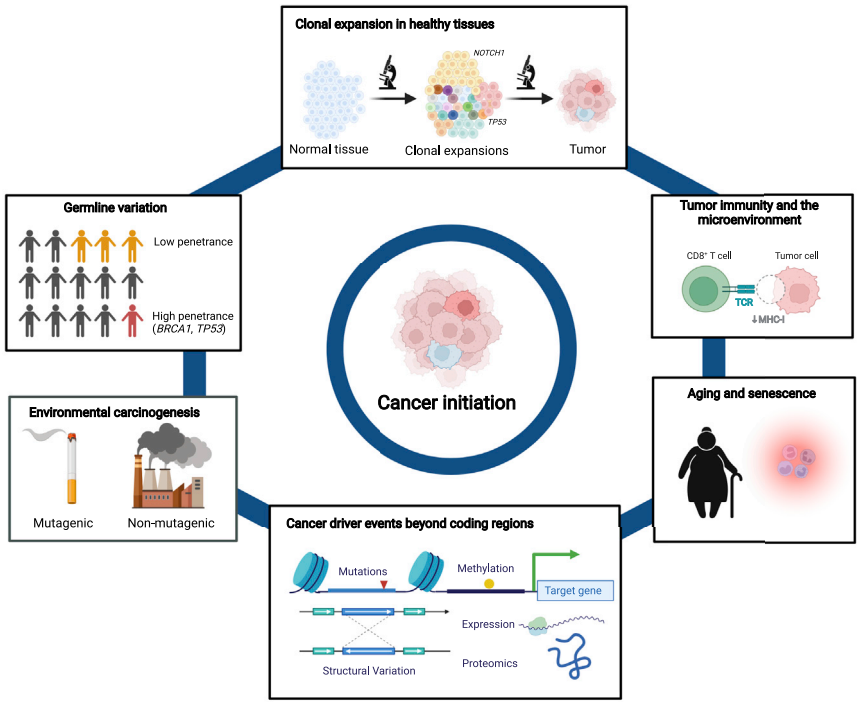

Figure 1. Future directions in cancer initiation research

In the cancer initiation field, key directions for research include (1) The relationship between somatic evolution and tumorigenesis from mutational landscape to selection and order of events (Clonal expansion in healthy tissues), (2) Paired germline and tumor specific studies to understand the impact of germline variation on cancer initiation (Germline variation), (3) Understanding the immune surveillance of somatic clones and its relationship with clone size, evolutionary trajectory and driver landscape (Tumour immunity and the microenvironment), (4) The relationship between ageing, senescence, somatic evolution and tumorigenesis (Ageing and senescence), (5) Mapping non-coding driver events across tumour types and quantifying non-genomic methods of selection (Cancer driver events beyond coding regions), and (6) Understanding the processes of mutagenic and non-mutagenic environmental carcinogenesis and the effect of chronic inflammation on cancer initiation (Environmental carcinogenesis).

sequencing (Bailey et al., 2018; McLendon et al., 2008; Parsons et al., 2008; Sjöblom et al., 2006) revealed that the coding regions of the tumor genome harbored from tens of point mutations in acute myeloid leukemias to thousands of mutations in melanomas (Lawrence et al., 2013). Mutational processes acting from embryological development onwards (Stratton et al., 2009) were found to fuel clonal evolution by generating variation in the tumor cell population, with a fraction of these mutations causing somatic alterations in driver genes that result in positive selection (Greaves and Maley, 2012; Nowell, 1976).

Analysis of point mutations and short insertions and deletions in 9,423 exomes from 33 tumor types in the Cancer Genome Atlas (TCGA) revealed 229 genes under positive selection, including *TP53* in ~80% (27/33) of cancer types, followed by *PIK3CA* (17) and *KRAS* (16; Bailey et al., 2018). Recently, analysis of 28,000 tumors across 66 tumor types uncovered 568 mutational drivers (Martínez-Jiménez et al., 2020). Other key studies have focused on the characterization of copy number (Zack et al., 2013), structural variation (Li et al., 2020b), methylation (De Carvalho et al., 2012; Pan et al., 2021) and gene fusions (Yoshihara et al., 2015), revealing alternative drivers of tumorigenesis. Alterations through these mechanisms include focal amplifications of *EGFR* in glioblastoma multiforme (GBM) (McLendon et al., 2008), *BRCA1* methylation in breast cancers (Esteller et al., 2000), and *EML4-ALK* fusions in non-small cell lung cancer (NSCLC) (Soda et al., 2007). The impact of large scale structural rearrangements, DNA copy number gains and losses and methylation events are far less well characterized than point mutations. What is clear is that most cancer-related genes are infrequently mutated across different cancer types.

Whole-genome sequencing has provided insights into the noncoding driver landscape of tumors (Elliott and Larsson, 2021; Rheinbay et al., 2020). For example, hotspot mutations in the *TERT* promoter, which increases telomerase expression and activity (Barthel et al., 2017; Rheinbay et al., 2020; Sabarinathan et al., 2017), affects 9% of all tumors within the Pan Cancer Analyses of Whole Genomes (PCAWG) consortium (Campbell et al., 2020), while other noncoding driver mutations were found to be relatively infrequent across cancer types (Elliott and Larsson, 2021). The future of noncoding driver analyses will benefit from technologies that can characterize the impact of mutations in regulatory regions (Mansour et al., 2014; Liu et al., 2020b) and the investigation of further cancer types.

The discovery of cancer-related events led to the concept of precision oncology, where treatments could be targeted to specific genomic alterations. Targeted therapies, such as imatinib in *BCR-ABL* mutant chronic myeloid leukemia (CML) (Druker et al., 2001), vemurafenib in *BRAF* V600E mutant melanoma (Chapman et al., 2011), gefitinib in NSCLC with activating *EGFR* mutations (Lynch et al., 2004), trastuzumab in *HER2*$^{+}$ breast cancer (Piccart-Gebhart et al., 2005), and exploiting synthetic lethality in *BRCA* mutated breast and ovarian cancers through poly (ADP-ribose) polymerase (PARP) inhibition (Fong et al., 2009) are well-known examples that have driven improvements in cancer outcomes. However, treatment strategies based on genotype-matched targeted therapy have yielded disappointing outcomes in recent studies, as typified by the National Lung Matrix Trial (NLMT) (Middleton et al., 2020a), LUNG-MAP (Redman et al., 2020), and NCI-MATCH (Salama et al., 2020) trials, in which over 13,000 patients with NSCLC were screened for actionable mutations in *HER2*, *FGFR1/2*, *MET*, *PIK3CA*, *PTEN*, *AKT*, *TSC1/2*, *KRAS*, *STK11*, *NRAS*, *BRAF*, *CCND1-3*, *CDK4*, *CDKN2A*, *ATM*, *ATR*, *BRCA1/2*, *PALB2*, and *NF1/2*. Collectively, across 37 genotype-matched cohorts involving 875 participants, the overall response rate was 7.5%, which is no different from the standard-of-care second-line chemotherapy docetaxel in NSCLC (Middleton et al., 2021).

It is clear that an actionable alteration in one tissue context may not be actionable in another. An example of this is the

contrast between vemurafenib monotherapy in *BRAF* V600E melanoma and colorectal cancer (CRC), where reported overall response rates range from 48% (Chapman et al., 2011) to less than 10% (Kopetz et al., 2015), respectively. Combining two or more treatments to target multiple actionable alterations has proven effective in certain cases. In metastatic *BRAF* V600E melanoma, combination therapy with a BRAF and a MEK inhibitor represents a standard-of-care treatment option (Keilholz et al., 2020); however, balancing efficacy with toxicity remains a barrier to widespread adoption of this strategy. Furthermore, the transcriptomic context of a tumor with an actionable alteration can be a determining factor in the treatment response to targeted therapy, as typified by the differential response to BRAF/MEK/epidermal growth factor receptor (EGFR) therapy between the *BRAF* V600E mutant (BM) transcriptional subtypes BM1 and BM2 in CRC (Middleton et al., 2020b). The relative paucity of actionable mutations and the impact of chromosomal instability on evolving cancer genomes have hindered progress in precision oncology.

Clonal expansions in healthy tissues

The process of cancer initiation begins with the healthy tissue. The concept that healthy tissue undergoes clonal expansion was substantiated through the observation of skewed chromosome X inactivation in the blood of healthy women (Busque et al., 1996). This was subsequently identified as a consequence of clonal hematopoiesis of indeterminate potential (CHIP), an aging-related clonal expansion of hematopoietic stem cells (Busque et al., 2012; Jaiswal et al., 2014).

The study of clonal evolution in healthy and pre-malignant solid tissues (Kakiuchi and Ogawa, 2021; Li et al., 2021; Moore et al., 2021) has utilized technologies such as sequencing of laser capture microdissected tissue (Ellis et al., 2021) and high-resolution duplex sequencing that accurately captures somatic mutations at low frequency (Abascal et al., 2021; Schmitt et al., 2012). Using these techniques, the process of small clonal expansions has been detailed across different tissues, including skin (Martincorena et al., 2015), colon (Lee-Six et al., 2019), esophagus (Martincorena et al., 2018; Yokoyama et al., 2019), bladder (Lawson et al., 2020), endometrium (Moore et al., 2020), liver (Brunner et al., 2019; Ng et al., 2021), pancreas (Li et al., 2021), and bronchus (Yoshida et al., 2020), with recent publications profiling several tissue types from the same individual (Li et al., 2021; Moore et al., 2021). Applying the same computational methods used to infer cancer driver genes (Martincorena et al., 2017) has uncovered genes, previously classified as cancer driver genes, under positive selection in non-cancerous tissue, including *NOTCH1* (skin, bronchus, and esophagus), *TP53* (esophagus and bronchus), and *PIK3CA* (endometrium and esophagus; Kakiuchi and Ogawa, 2021). Mutations found in RNA sequencing (RNA-seq) data from healthy individuals within the Genotype-Tissue Expression (GTEx) consortium also revealed clonal expansions in different tissues (García-Nieto et al., 2019; Yizhak et al., 2019). Somewhat different from somatic mutations, which have been virtually ubiquitously described in all tissues, DNA copy number aberrations were observed in less than 10% (37/389) of 389 samples across 29 different histologies (Moore et al., 2021). Interestingly, esophageal and cardiac tissues harbor more copy number alterations than other organ sites (Li et al., 2021).

A key question is how can ostensibly clear cancer-related driver somatic events exist in small populations of cells in histologically normal tissue? The cancer somatic driver landscape differs from the driver landscape in normal tissue, which suggests the presence of distinct selective pressures. One clear example is *NOTCH1*, commonly mutated in normal esophageal epithelium (~66%) but less frequently in esophageal squamous cell carcinoma (~15%; Yokoyama et al., 2019). *NFKBIZ* mutations are commonly found in clonal expansions from non-cancerous epithelium of patients with ulcerative colitis compared with colitis-related cancer. *NFKBIZ* mutations may confer a selective advantage in a chronically inflamed environment; however, these mutations may be negatively selected in cancer and restrict tumor formation (Kakiuchi et al., 2020). It has been proposed that ongoing clonal competition in normal epithelium can result in eradication of malignant cells (Colom et al., 2021). Fewer tobacco-related mutations and longer telomeres are detected in the healthy lung of ex-smokers compared with current smokers, suggesting that less affected cells expand after withdrawal of the mutagenic stimulus (Yoshida et al., 2020). In the intestinal crypts, however, it has been shown that *Apc*-mutant cells can outcompete wild-type clones through the secretion of WNT antagonists, such as NOTUM, resulting in the formation of adenomas (Flanagan et al., 2021).

Unraveling mechanisms that drive the clonal expansion of normal tissue toward early cancer initiation will inform cancer interception strategies. Understanding why cells are at risk of transformation following acquisition of a somatic driver event will be a key step in this process. Malignant transformation seems to be intimately linked to the microenvironment, cell of origin, and the underlying epigenetic and transcriptional program (Chang et al., 2016; Haigis et al., 2019; Jonsson et al., 2019). For example, the ability of *BRAF* V600E to drive tumor initiation was evident in the neural crest and melanoblast lineages but less so in the melanocyte lineage in zebrafish (Baggiolini et al., 2021). Single-cell lineage tracing and transcriptomic analyses in mice have shown the propensity for malignant transformation in cells with a *BRAF* V600E mutation is affected by the spatial context of the cell and the tissue of origin (Köhler et al., 2017; Moon et al., 2017). The timing of the acquisition of mutations also influences disease phenotype; for example, the order of *TET2* and *JAK2* mutations influence the type, onset, and treatment sensitivity of myeloproliferative disorders (Ortmann et al., 2015).

Genomic technologies have revealed the pervasive nature of mutations capable of driving tumorigenesis across tissues, and future discoveries will enhance our understanding as to which local and systemic factors trigger both malignant and non-malignant clonal expansions across these genetic backgrounds.

Mutagenic and non-mutagenic causes of environmental carcinogenesis

The association between cancer initiation and environmental carcinogens has been long established experimentally (Auerbach and Robson, 1946); however, during the past decade, the use of mutational signatures to describe both exogenous and endogenous mutational processes has strengthened the assertion of causal links between environmental exposures and their mutational footprints (Alexandrov et al., 2013, 2020; Kucab et al., 2019; Zou et al., 2018). Exogenous mutational

processes, including UV light (mostly causing C to T transitions, C > T, signature 7; Tessman et al., 1964), smoking (C > A, signature 4; Alexandrov et al., 2016), alcohol consumption (T > C, signature 16; Chang et al., 2017; Letouzé et al., 2017), aristolochic acid (T > A, signature 22; Hoang et al., 2013; Ng et al., 2017), platinum-based drugs (C > A and C > T, signatures 31 and 35; Boot et al., 2018; Pich et al., 2019), and *pks*$^{+}$ *E. coli* (T > G, signature 88; Pleguezuelos-Manzano et al., 2020) suggest that, in some cases, the link between cancer incidence and environmental exposures is related to the generation of somatic mutations. It is likely, for example, that *KRAS* G12C mutations in NSCLC are generated through smoking-related mutagenesis (Muiños et al., 2021; Temko et al., 2018). It is also possible that environmental factors exacerbate some endogenous mutational processes, for example, increased APOBEC mutagenesis (C > T and C > G mutations, signatures 2 and 13) after irradiation (Saito et al., 2020).

Despite this link, many environmental exposures initiate tumorigenesis in ways that appear to be non-mutagenic. In Riva et al. (2020), 17/20 known suspected carcinogens in mice increased tumorigenesis but did not increase mutational burden or generate a specific mutational process. Furthermore, the Mutograph project revealed no distinct mutational patterns in esophageal cancers that could explain the international geographical disparities in cancer incidence (Moody et al., 2021). One plausible cause of non-mutagenic carcinogenesis is epigenetic aberrations, which might deregulate expression of cancer-related genes (Black and McGranahan, 2021; Hanahan and Weinberg, 2011). Several metals, including lead and arsenic, can cause oxidative damage that hampers the interaction between methyltransferases and the DNA, ultimately altering the methylation landscape of the cell, which can lead to tumorigenesis (Baccarelli and Bollati, 2009; Zhao et al., 1997). Smoking also seems to affect the methylation landscape in lung cancers; however, this is not observed in other tissues exposed to tobacco, such as pharyngeal or oral cancers (Alexandrov et al., 2016). Particulate matter seems to have a moderate impact on methylation patterns in leukocytes (Tarantini et al., 2009). It is also possible that different environmental exposures might lead to alterations in selection pressures that permit malignant clonal expansion, a phenomenon described in acute myeloid leukemias after exposure to chemotherapy (Pich et al., 2021; Wong et al., 2015).

Environmental exposures may also facilitate malignant transformation through chronic inflammation. The incidence of liver, esophageal, and pancreatic cancer increases with alcohol consumption (Wang et al., 2010), and the link between mesothelioma and asbestos exposure is well established (Qi et al., 2013). The association between lung cancer and air pollution may also be driven by inflammation (Lim et al., 2012; Raaschou-Nielsen et al., 2010, 2013). Understanding the non-mutagenic mechanisms of environmental carcinogenesis will in the future incorporate cell-intrinsic processes, such as epigenetic alterations, and extrinsic processes that may directly affect the tumor microenvironment, permitting clonal expansions and transformation.

Immune surveillance, aging, and senescence

Tumor growth is constrained by an active immune system (Hanahan and Weinberg, 2011). Consequently, cancer cells resilient to immune surveillance gain a selective advantage in a process known as immunoediting. This process is facilitated by tumor-intrinsic immune escape events, including loss of *HLA* alleles, and mediated by the tumor microenvironment (McGranahan et al., 2017). It is unknown when immune surveillance affects expanding clones in normal tissues. It does not appear that microscopic clonal expansions brought about by somatic mutations in normal tissue elicit a strong immune response (Li et al., 2021; Moore et al., 2021). However, the detection of immune infiltrates in pre-invasive lung adenocarcinoma (Chen et al., 2019) and squamous cell carcinoma (Pennycuick et al., 2020), with coinciding putative immune escape events, such as *HLA* loss of heterozygosity and in squamous cell carcinoma *HLA* promoter hypermethylation, implies that there is a point where an expanding non-malignant clone triggers detection. The increased incidence of malignancies, such as Kaposi sarcoma, lymphomas, and cancers of the stomach, lung, liver, oropharynx, and cervix, in patients with HIV suggests the requirement for ongoing immune surveillance to prevent tumor initiation and progression, in particular (but not limited to) cancers related to viral infections (Grulich et al., 2007).

The incidence of cancer is intimately related to aging, increasing from 25 cases per 100,000 in those less than 20 years old to more than 1,000 per 100,000 in those over the age of 60. Genomic instability, telomeric dysfunction, epigenetic alterations, and cellular senescence are all damaging cellular processes that increase with age (López-Otín et al., 2013). Cells also accumulate mutations with aging, the most common process being spontaneous 5-methylcytosine deamination, which leads to C > T mutations at CpG sites (signature 1; Alexandrov et al., 2015; Moore et al., 2021). Most driver mutations can be attributed to this age-related mutational process (Muiños et al., 2021); however, this temporal coincidence occurs independent of the number of driver mutations themselves (Rozhok and DeGregori, 2019).

Senescence, a process whereby proliferation is arrested in response to cellular stresses, is implicated in the association between aging and cancer (Fane and Weeraratna, 2020). This phenomenon involves a senescence-associated phenotype (SASP), which includes secretion of pro-inflammatory cytokines, growth factors, and proteases that can affect neighboring cells (Coppé et al., 2010; Rodier et al., 2009). This sustained and systemic low-grade chronic inflammation is one of the hallmarks of aging and is termed "inflammaging" (López-Otín et al., 2013). The number of senescent cells increases exponentially with aging (Dimri et al., 1995; Herbig et al., 2006), and it has been shown that depletion of senescent cells from middle-aged mice delayed cancer progression (Baker et al., 2016). The SASP has been shown to suppress CD8^{+} T cell activity through the recruitment of myeloid-derived suppressor cells and regulatory T (Treg) cells in mouse models, diminishing immune surveillance and thus promoting the emergence of neoplastic clones (Ruhland et al., 2016).

Age also has a detrimental effect on the immune system (Fane and Weeraratna, 2020). *In vivo* experiments have shown that the induction of early-onset senescence in hematopoietic cells causes impaired innate and adaptive immune function, in particular, of natural killer and follicular helper T cells (Yousefzadeh et al., 2021). Senolytic therapy, the process of selectively

inducing apoptosis in senescent cells, is an intriguing concept as adjuvant tumor therapy (Short et al., 2019; Wang et al., 2022).

The influence of germline variation in cancer initiation

Sequencing the germline of patients with suspicion of hereditary syndromes linked to an increased cancer incidence has revealed recurrently affected genes, including *BRCA1* and *BRCA2* in hereditary breast and ovarian cancer (HBOC) syndrome and *TP53* in Li-Fraumeni syndrome. In a recent study, the profile of 17,152 prospectively sequenced patients across 55 tumor types using the MSK-IMPACT panel (which targets 341 cancer-related genes) revealed that ~7.8% patients harbored pathogenic germline variants, with *BRCA1* and *BRCA2* affecting more than 2% of the entire cohort (Srinivasan et al., 2021). However, the role of the majority of these germline variants is unclear and the pathogenicity of some may be conditioned by the cell of origin (Jonsson et al., 2019; Srinivasan et al., 2021). These highly penetrant mutations are rare in the general population, although it is expected that the burden of low-penetrance germline variants that increase cancer risk is much higher (Sud et al., 2017). Mosaic mutations acquired in early embryogenesis affecting cancer-related genes, including *TP53* and *RB1*, were also found to impact cancer development in 0.1% of patients (Pareja et al., 2021).

The interaction between the cancer cell and the microenvironment can also be modulated by germline variation. Specific germline variants, including those affecting *STING1*, *TMEM108*, *IFIH1*, and major histocompatibility complex class I (MHC class I) and MHC class II genes, can have an impact on antigen presentation, immune infiltration, and immunotherapy responses (Chowell et al., 2019; Marty et al., 2017; Marty Pyke et al., 2018; Naranbhai et al., 2022; Pagadala et al., 2021; Sayaman et al., 2021; Shahamatdar et al., 2020).

Genome-wide association studies have provided more than 420 cancer associations at 262 genomic loci (Sud et al., 2017), with only 5% located in the coding region. A few of these variants have been linked to susceptibility to systemic and environmental exposures, including one intronic SNP at the cholinergic nicotinic receptor subunit alpha 3–5 (*CHRNA3–CHRNA5)* gene, which is associated with lung cancer through nicotine addiction, increased smoking, and difficulties in quitting (Amos et al., 2008; Freathy et al., 2009; Sud et al., 2017; Thorgeirsson et al., 2008).

Genomic technologies have allowed the profiling of germline variation of hundreds of thousands individuals (Bycroft et al., 2018; Taliun et al., 2021). Larger germline analyses in cancer patients together with detailed tumor characterization, mosaicism, clinical histories, and environmental exposures will reveal new variants linked to cancer susceptibility and in which context they act. In turn, this will provide new tools for cancer prevention and patient stratification and perhaps will bring the implementation of polygenic risk scores into cancer-screening programs (Adeyemo et al., 2021; Khera et al., 2018).

CANCER PREVENTION AND EARLY DETECTION

Cancer prevention is key to reducing cancer risk, and early detection is key to improving cancer outcomes. Here, we discuss mitigation of risk through primary prevention, touching on chemoprevention and cancer vaccines, interception, and screening approaches, including the use of circulating tumor DNA (ctDNA) in early detection. We outline some of the key areas for translational research in Figure 2.

The aim of primary prevention is to reduce cancer incidence. This can be achieved through the reduction of causative exposures, such as through human papillomavirus (HPV) vaccination (Falcaro et al., 2021), or through prophylactic intervention following identification of high risk individuals, for example, risk-reducing mastectomy for carriers of *BRCA1* or *BRCA2* mutations (Collins, 1996). For other cancer-predisposition syndromes, the focus of management remains on early detection. High-throughput sequencing is facilitating broader access to germline testing in the clinic (Richards et al., 2015), resulting in vast amounts of information concerning genetic variants in the population, leading to a refinement of prevention and targeted treatment strategies. However, many variants do not have predictable phenotypic consequences and are labeled variants of uncertain significance (VUS). Approaches such as saturated genome editing (Findlay et al., 2014, 2018) provide functional evidence of the consequences of rare germline mutations, which can improve the predictive accuracy of the information provided to patients.

Chemoprevention

Chemoprevention, the broad use of medication to prevent disease, is a common strategy used outside of the cancer field, for example, the administration of antihypertensives and reduction in low-density lipoprotein (LDL) cholesterol synthesis used to reduce the incidence of cardiovascular disease. This has also been utilized in the cancer field. The International Breast Cancer Intervention Study (IBIS) I trial found that tamoxifen reduced breast cancer incidence in women deemed to be at high risk of cancer development by a third (Cuzick et al., 2002), even after treatment cessation (Cuzick et al., 2015). The Mammary Prevention 3 (MAP.3) (Goss et al., 2011) and IBISII (Cuzick et al., 2014, 2020) clinical trials have reported a relative risk reduction in breast cancer incidence of 65% and 49% after treatment with exemestane and anastrozole, respectively. However, adverse effects associated with exposure to tamoxifen (endometrial cancers and venous thromboembolism) and aromatase inhibitors (bone fractures) have restricted uptake of chemoprevention drugs outside clinical trials (Smith et al., 2016).

There is evidence that chemoprevention with 5-alpha-reductase inhibitors may reduce the incidence of prostate cancer (Andriole et al., 2010; Thompson et al., 2003), and cyclo-oxygenase (COX) 1 and 2 inhibitors, such as aspirin, lower the risk of distant metastasis and increase survival in CRC (Liao et al., 2012; Rothwell et al., 2012). Furthermore, from a pooled analysis of two cohort studies involving 94,540 patients, aspirin use initiated before 70 years of age was found to reduce the incidence of CRC (Guo et al., 2021), likely through the interaction between prostaglandin metabolism, WNT signaling pathway regulation, and chronic inflammation (Drew et al., 2016). Breakthroughs in chemoprevention will stem from a deeper understanding of clonal competition in normal epithelium and the impact of the tissue microenvironment and environmental exposures upon this process, including cancer initiation.

Vaccines for prevention

Educating the immune system to eliminate pre-malignant lesions has been successful in cancers driven by viral infection.

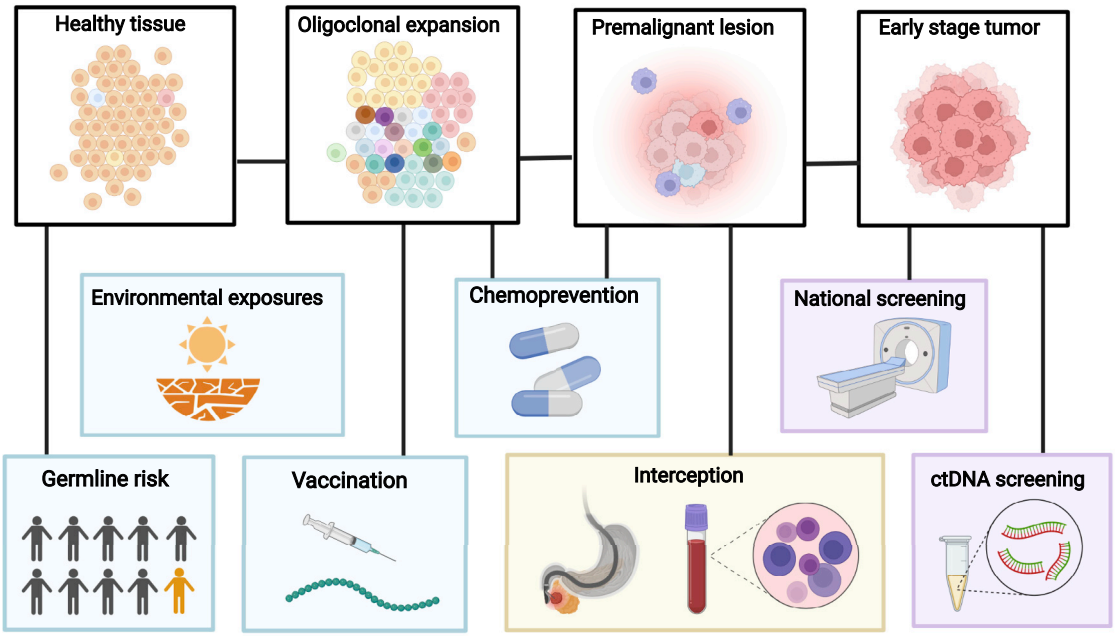

Figure 2. Key areas for translation in cancer prevention and early detection

In cancer prevention and early detection, key areas include (1) Risk stratification of individuals who harbor germline variants that increase susceptibility to cancer (Germline risk), (2) Reduction of environmental exposures, including mitigating the effects of climate change (Environmental exposures), (3) Identification of tumor-specific neoantigens or common oncogenic mutations as vaccine targets (Vaccination), (4) The use of preventative medication in high-risk individuals to reduce cancer incidence (Chemoprevention), (5) Methods to better characterize and detect pre-malignant tissue to determine risk of malignancy and further development of intervention strategies (Interception), (6) Adoption of enhanced screening protocols for high-risk individuals (National screening), and (7) The use of circulating tumor DNA (ctDNA) to detect early-stage malignancies and the development of methods to detect low-burden disease (ctDNA screening).

Vaccines based on viral antigens from the HPV not only reduced the incidence of cervical cancer (Falcaro et al., 2021; Kenter et al., 2009) but have also shown potential to control pre-malignant clonal expansions (Trimble et al., 2015). The success of hepatitis B vaccination in lowering the incidence of hepatocellular carcinoma is well documented (Chen, 2009).

Beyond vaccines based on viral antigens, tumor-specific neoantigens are the targets of effective tumor-immune responses (Blass and Ott, 2021). Cancer neoantigens encoding peptides with a strong MHC class I binding affinity are ideal candidates for vaccine targets, can be predicted computationally, and require histocompatibility leukocyte antigen (HLA) class I typing from individual patients. There are a number of ongoing trials of personalized neoantigen vaccines in patients with established solid tumors (NCT03633110, NCT03289962, and NCT03313778); however, despite excellent T cell responses, objective response rates remain modest. Broadening the therapeutic potential of cancer neoantigen vaccines to the prevention setting (Crews et al., 2021) to target clonal, cancer-initiating mutations in high-risk populations, such as peptide vaccines to *KRAS* G12C in heavy smokers, may reap future benefits.

Interception

Interception lies at the interface of cancer prevention and early detection and includes the process of identifying pre-malignant cells or tissue with the goal of preventing tumor formation as part of a cancer prevention strategy. Through genomic profiling of pre-malignant tissue, important insights into early carcinogenesis have been revealed. One example is Barrett's esophagus (BE), a gastroesophageal-reflux-related precursor lesion to esophageal adenocarcinoma (EAC) characterized by metaplasia of the epithelium (Spechler, 2013). Patients will have a risk of progressing from low-grade dysplasia to EAC of 0.3% per year (Hvid-Jensen et al., 2011). There have been developments in minimally invasive strategies to detect high-risk individuals in the general population. This is exemplified by the Cytosponge-trefoil factor 3 (TTF3), a test based on a non-endoscopic device to detect dysplasia. The BEST3 clinical trial was conducted among 109 general practices in England in patients over 50 years old with a minimum 6-month history of gastroesophageal reflux. After 12 months of follow-up, the rate of BE detection among those randomized to the Cytosponge-TFF3 was 10-fold that of the standard of care whereby patients only received an endoscopy if requested by their general practitioner (Fitzgerald et al., 2020).

Lung squamous cell carcinoma is often preceded by pre-malignant lesions in the bronchial airways, especially in the context of smoking (Auerbach et al., 1961). Similarly, adenomatous hyperplasia in the lung can progress to invasive adenocarcinoma (Weichert and Warth, 2014). Molecular characterization has revealed differences between pre-malignant regions that spontaneously regress and those that progress to invasive disease over time. Progressive lesions harbored evidence of greater genomic instability and immune escape events, including *B2M* mutations and HLA loss of heterozygosity, compared with regressive lesions. Interestingly, regressive lesions had epigenetic and transcriptomic profiles closer to normal bronchial epithelium with increased $CD8^+$ T cell infiltration defined by

RNA-seq and histopathology (Pennycuick et al., 2020; Teixeira et al., 2019).

There has been progress in the understanding of the biology of pre-malignant lesions and development of methods to detect them. In cases such as cervical adenocarcinoma *in situ* (AIS) and BE, there are also established and promising interception strategies, such as cold knife conization or loop electrosurgical excision for cervical AIS (Teoh et al., 2020) and endoscopic submucosal resection for BE (Pech et al., 2014). Initiatives such as the Precancer Genome Atlas (funded by the US National Cancer Institute; Srivastava et al., 2018) aim to profile pre-malignant samples across different organs with both imaging and genomic technologies. As our understanding of clonal expansions in pre-malignant and normal tissue develops, strategies to identify early high-risk lesion development and management will improve. Sampling healthy tissue from most organs is often invasive; therefore, there is an unmet need to establish programs that utilize the sampling of normal tissue following surgery or routine procedures, such as endoscopies.

Early detection

The aim of early detection is to reduce the proportion of patients diagnosed with cancer at a late stage to maximize the probability of cure (Hawkes, 2019). For many cancers, such as lung, breast, and CRC, this is a crucial aspect of cancer control. In the UK, for CRC, the 1-year net survival of patients diagnosed at stage 1 was 97.7% compared with 43.9% at stage 4 between 2013 and 2017. For lung cancer, 1-year net survival at stage 1 was 87.7%, and at stage 4, it was 19.3%. For breast cancer, this was 100% at stage 1 and 66% at stage 4 in the same time period (Office for National Statistics, 2019). There is strong evidence that screening programs reduce cancer mortality for patients diagnosed with these cancer types. A systematic review of four clinical trials (Kronborg et al., 2004; Lindholm et al., 2008; Mandel et al., 2000; Scholefield et al., 2002) estimated that the risk reduction of CRC mortality was 15% in studies that screened twice yearly (Hewitson et al., 2007). The Dutch-Belgian Lung Cancer Screening Trial (NELSON) of 13,195 male participants reported a rate ratio for death from lung cancer of 0.78 comparing computed tomography (CT) screening at baseline and after 1 year, 3 years, and 5.5 years with no screening (de Koning et al., 2020). The US National Lung Screening Trial (NLST) also reported a relative reduction in lung cancer mortality of 20% in a trial of 54,454 participants (Team, 2011). For breast cancer, results of a meta-analysis of 11 randomized control trials estimated that the relative risk reduction of breast cancer mortality was 20% (Independent UK Panel on Breast Cancer Screening, 2012), with recent evidence from the UK age trial concluding that reducing the screening age of women by 10 years (to 40 years old) yields further reduction in breast cancer mortality with minimal impact on overdiagnosis (Duffy et al., 2020).

The benefit of early detection is attenuated by the low positive predictive value of the test, investigation and treatment of false-positive results, and over-treatment of indolent cancers. This has been keenly debated in the breast cancer field (Paci et al., 2014), and targeted screening of high-risk individuals or personalized screening strategies based on individual risk will continue to be developed (Louro et al., 2021).

The use of high-throughput genomic assays to facilitate early detection is beginning to offer complementary approaches to cancer screening, primarily using ctDNA. ctDNA is the tumor-specific fraction of cell-free DNA (cfDNA), extracellular DNA that is released into the plasma. ctDNA has great potential as a minimally invasive tumor biomarker, and there are numerous studies demonstrating that the amount of ctDNA detected in the plasma correlates with tumor burden, metabolism, and rate of proliferation (Abbosh et al., 2017; Bredno et al., 2021; McEvoy et al., 2018). Examples in the field include the CancerSEEK and Galleri assays. The CancerSEEK assay combines ctDNA detection of tumor-specific mutations with protein biomarkers. In 2018, Cohen et al. (2018) used CancerSEEK to study 1,005 patients with eight tumor types of stages I–III, with the assay detecting mutations in 1,933 distinct genomic positions and eight blood-based biomarkers: cancer antigen 125 (CA-125), carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA19-9), hepatocyte growth factor (HGF), myeloperoxidase (MPO), osteopontin (OPN), prolactin (PRL), and tissue inhibitor of metalloproteinases 1 (TIMP-1). The CancerSEEK assay was the basis of the DETECT-A feasibility study, a prospective study of 10,006 women, which combined the blood tests with a diagnostic positron emission tomography (PET)-CT (where positive), to confirm and localize the site of disease (Lennon et al., 2020). Of 96 incident cancers, 26 were picked up through blood testing, from which 15 underwent PET-CT and nine had surgery with curative intent. Promisingly, the combined testing approach improved the sensitivity of the blood test alone from 98.9% to 99.6% and the positive predictive value from 19.4% to 28.3%. The risk-benefit and the clinical utility (i.e., does the test reduce cancer mortality) of this type of approach is to be determined.

Also in 2018, through the Circulating Cell-free Genome Atlas (CCGA) study (Liu et al., 2018; Oxnard et al., 2018), three ctDNA-based sequencing assays were compared that represented differing approaches to detection: through targeted panel sequencing of single nucleotide variants and indels (targeted panel), whole-genome sequencing for copy number variation (WGS-CNV), and whole-genome bisulfite sequencing (WGBS) for DNA methylation patterns with the aim of developing a multi-cancer early detection (MCED) test. It was shown that ctDNA detection through methylation patterns provided the highest sensitivity across multiple-stage cancer types; among 63 stage I–IIIA patients with NSCLC, the sensitivity was 48%, 54%, and 56% for the targeted panel, WGS-CNV, and WGBS, respectively.

Following this, a targeted MCED approach of >100,000 informative methylation regions was then evaluated among 6,689 participants (including 2,482 cancer patients in >50 cancer types). In a pre-specified set of 12 cancer types, sensitivity was 39% for stage I, 69% for stage II, 83% for stage III, and 92% for stage IV, at a specificity of >99% (Liu et al., 2020a). Through a machine-learning classifier, determination of tissue of origin of the ctDNA signal was also >90% (Liu et al., 2020a). This multi-cancer early detection approach formed the basis of the Galleri assay, developed by GRAIL, from which the prospective NHS-Galleri trial has been established in the UK. The trial is randomizing 140,000 participants between 50 and 77 years to the Galleri assay or observation to assess whether the assay can be used to shift cancer diagnoses to early disease stages.

Beyond methylation profiling, fragmentomics and topological analyses are alternative approaches to ctDNA analysis (Lo et al., 2021). Fragmentomics analyzes the product of differential enzymatic fragmentation in tumor and non-tumor cfDNA, in the form of plasma DNA end motifs (Jiang et al., 2020). Topological analysis includes the identification of circular DNA structures, such as extrachromosomal circular DNA (eccDNA) (Zhu et al., 2017).

The promise of ctDNA assays in early detection may be further enhanced by our understanding of ctDNA kinetics and the relationship of ctDNA fraction that may complement conventional pathological tumour staging. This understanding may identify patients with tumors that are more likely to recur following surgical intervention and in whom neoadjuvant and adjuvant treatment can be tailored accordingly.

ADJUVANT THERAPY AND MINIMAL RESIDUAL DISEASE MONITORING

Minimal residual disease (MRD) monitoring refers to the process of detecting and quantifying minute populations of cancer cells that may persist post treatment. The application of next-generation sequencing approaches to MRD monitoring has led several translational studies to evaluate clinical utility in the adjuvant therapeutic setting, most notably through ctDNA detection. In this setting, patients at high risk of recurrence are identified through post-surgical MRD detection. This approach may improve the stratification of patients who may benefit from adjuvant therapy and potentially avoid unnecessary treatments in those at low risk of disease recurrence. Moreover, the increasing sensitivity of MRD testing has facilitated the detection of recurrence months before imaging or biopsy-confirmed relapse.

Using a phylogenetic approach to MRD monitoring through the detection of clonal and subclonal SNVs using a multiplex PCR panel, we were able to detect the emergence of metastatic subclones with a median lead time of 70 days prior to imaging recurrence (Abbosh et al., 2017). The "personalized" approach of utilizing tumor-specific mutations, developed through Tracking Cancer Evolution through Therapy (TRACERx) collaborative work, is used for the Signatera assay, which has been employed in a number of trials to determine how serial ctDNA monitoring can be used as a predictive biomarker in patients receiving checkpoint inhibition (CPI). The MERMAID-1 trial will assess the efficacy of durvalumab combination with chemotherapy in resected stage II–III NSCLC in those who are MRD positive (NCT04385368). In the adjuvant setting, among 94 patients diagnosed with multiple tumor types as part of the INSPIRE trial, baseline ctDNA concentration correlated with clinical response, disease-free survival, and overall survival in patients treated with pembrolizumab (Bratman et al., 2020; Powles et al., 2021). Furthermore, as a preplanned retrospective analysis of the IMvigor010 trial, Powles and colleagues demonstrated that patients with detectable ctDNA had improved disease-free survival and overall survival from atezolizumab in a trial of 581 patients who had undergone surgery for operable urothelial carcinoma (Powles et al., 2021).

Tracking tumor mutations in the context of low tumor burden and at low allele frequencies presents a challenge. Zviran et al. (2020) adopted an alternative approach (MRDetect) to targeted deep sequencing through whole-genome sequencing of cfDNA. MRDetect utilizes the cumulative signal of thousands of tumor mutations as priors to enhance the detection threshold of variants at both low allele frequencies and ctDNA fraction (Zviran et al., 2020). By utilizing phased variants derived from whole-genome-sequenced tumor samples (PhasED-seq), Kurtz et al. (2021) were able to improve the sensitivity of ctDNA detection in diffuse large B cell lymphoma, leveraging the fact that detection of two or more mutations that occur in *cis* reduces the background error.

Beyond early detection and MRD monitoring, the utility of ctDNA in selecting patients for mutation-directed therapy was assessed in the plasmaMATCH trial, a multicenter phase 2a platform trial of 1,051 patients with advanced breast cancer (Turner et al., 2020). Patients with a targetable mutation in *PIK3CA*, *ESR1*, *HER2*, and *AKT1*, confirmed through digital droplet PCR and the Guardant 360 targeted sequencing panel, were subsequently offered entry into one of four treatment arms. Agreement for gene-level mutational status between the two assays was 96%–99%, with sensitivity of ctDNA digital droplet PCR and targeted panel sequencing compared with contemporaneous tumor biopsies at 98% (95% confidence interval [CI] 87–100) and 100% (92–100), respectively. This provides a strong argument for the clinical utility of ctDNA in identifying targetable mutations in the advanced-stage disease setting (Turner et al., 2020).

ctDNA monitoring has highly promising potential in the neoadjuvant setting, and may be employed across multiple tumor types in the future. We expect progress in the adjuvant setting to be accelerated using MRD biomarkers to switch therapies in the absence of disease on imaging with the potential to set up multi-arm trial adjuvant MRD programs where therapy could be switched if ctDNA fractions rise on treatment. MRD assays, by identifying patients who are at greatest need of adjuvant therapy intervention, also offer significant potential to reduce the size, cost, and time taken to conduct and report adjuvant trials.

DRUG RESISTANCE

Tackling drug resistance is perhaps the biggest challenge to achieving cancer cures in the advanced-disease setting. The nature of drug resistance is multifaceted, with several interdependent key biological determinants that variably affect resistance to chemotherapy, targeted therapy, and immunotherapy. These facets can be broadly divided into intratumor heterogeneity, adaptive responses to targeted therapeutic pressures, cancer immunogenicity and the impact of the tumor microenvironment, and physical constraints to intratumoral delivery of drug (Vasan et al., 2019). These facets are further complicated by the current panoply of undruggable targets, typified by transcription factors and tumor-suppressor proteins. Here, we explore contributions to four of those key determinants (Figure 3) and address the broad areas for future research.

Intratumor heterogeneity

As cancers evolve, somatic mutations accumulate through temporally and spatially distinct mutational processes. This creates genetic diversity, and a subset of these mutations may confer a fitness advantage which drives clonal expansions of cancer cells. These clonal expansions contribute to the genetically distinct subclonal populations of cancer cells that underlie the intratumor heterogeneity (ITH) observed across cancer types through bulk multi-region sequencing and single-cell sequencing

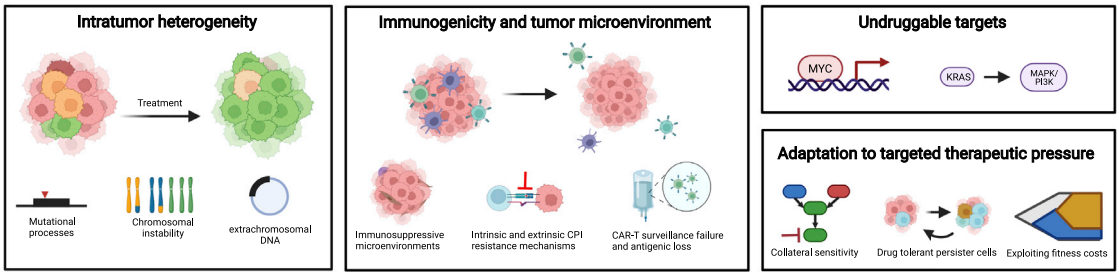

Figure 3. Selected key areas in the drug resistance field

Genetic and non-genetic intratumor heterogeneity (ITH) is the variation from which Darwinian evolution and clonal selection of drug-resistant phenotypes may occur. Understanding drivers of ITH, targeting molecular vulnerabilities, and enhancing immune responses to chromosomally unstable tumor cells offer routes to forestall drug resistance (Intratumor heterogeneity). Stimulating tumor immune responses through checkpoint inhibition (CPI) and adoptive T cell therapies (including CAR-T cells) have delivered success in attenuating tumor progression. Mechanisms of CPI and CAR-T resistance have been well characterized, from which strategies to remodel the tumor microenvironment, prevent antigenic loss, and optimize T cell function will continue to develop (Immunogenicity and tumor microenvironment). The success of targeting clonal driver alterations, such as KRAS G12C, may herald the development of further therapies toward targets previously considered "undruggable" (Undruggable targets). Tumors can adapt rapidly to targeted therapeutic pressure through non-genetic feedback, including pathway switching and epigenetic modulation, or acquire resistance through mechanisms such as oncogene amplification, splice variants, or driver mutations. Collateral sensitivity is a process where acquired resistance to a specific therapy results in increased therapeutic vulnerability to a different therapy, due to resistance pathways that often converge on conserved downstream signalling pathways. Drug-tolerant persister cells enter a reversible epigenetic-driven phenotype that arrests or proliferates slowly under therapeutic pressure. Acquiring resistance mechanisms may come at a fitness cost if they affect important cellular functions. This could be exploited when therapeutic pressure is removed, leading to emergence of drug-sensitive clones that outcompete drug-resistant clones (Adaptation to targeted therapeutic pressure). Understanding the interplay between these mechanisms will lead to further approaches to tackling targeted therapy resistance.

approaches (Gerlinger et al., 2012; Marusyk et al., 2020; McGranahan et al., 2017). ITH is known to contribute to poor outcomes and drug resistance (Greaves, 2015) and provides genetic and non-genetic diversity upon which clonal selection may act (Black and McGranahan, 2021; Turati et al., 2021).

ITH at the single-nucleotide scale is driven by the mutational processes active in the tissue, which includes age-related spontaneous deamination and APOBEC cytidine deamination, which often occurs later in tumor evolution (Petljak et al., 2019; de Bruin et al., 2014; Jamal-Hanjani et al., 2017). Structural variants (SVs) and somatic copy-number alterations (SCNAs) are thought to occur as a consequence of chromosomal instability (CIN): the occurrence and tolerance of chromosome segregation errors during cell division (Bakhoum and Cantley, 2018; Sansregret et al., 2018), resulting in aneuploidy where the karyotype of the cell is not a multiple of the haploid component. Large-scale macro-evolutionary events, typified by whole-genome doubling (WGD), have an extensive impact on the genome, and WGD is associated with both increased SCNA heterogeneity (Dewhurst et al., 2014; Watkins et al., 2020) and poor prognosis across cancer types (Bielski et al., 2018).

The mutational landscape has been shown to change after treatment with both chemotherapeutics (Ding et al., 2012; Johnson et al., 2014; Murugaesu et al., 2015; Schuh et al., 2012) and targeted therapies (Bettegowda et al., 2014; Diaz et al., 2012; Misale et al., 2014; Shah et al., 2012; Shi et al., 2014), often reflecting selection of pre-existing resistant clones. However, genotoxic agents, such as platinum-based chemotherapeutics, topoisomerase inhibitors, and radiotherapy, possess the potential to generate DNA damage (Pich et al., 2019; Pilger et al., 2021) and may cause genomic instability and somatic mutations (Boot et al., 2018; Pich et al., 2019; Pilger et al., 2021), generating further ITH.

Identifying vulnerabilities particular to chromosomally unstable and WGD cells holds promise through approaches such as KIF18A inhibition. KIF18A, a microtubule-associated kinesin, was identified as important in WGD cells through mitotic kinesin knockdown assays comparing chromosomally stable and unstable cell lines (Marquis et al., 2021), essentiality data from cancer cell lines (Quinton et al., 2021), genomics data from Cancer Cell Line Encyclopedia (CCLE) (Ghandi et al., 2019), and genetic screening analysis (Cohen-Sharir et al., 2021).

ITH can also be supplemented through extrachromosomal DNA (ecDNA). These are circular genomic SVs that often harbor oncogenes but do not contain centromeres and so are subject to random segregation during metaphase: contributing to extreme SCNA amplification, cell-to-cell copy number variation, high oncogene expression, and genomic remodeling (Bailey et al., 2020; Koche et al., 2020; Verhaak et al., 2019; Wu et al., 2019).

ecDNA has been shown to drive targeted therapy resistance in EGFRvIII mutant (deletion from exon 2–7) glioblastoma through chromosomal reintegration (Nathanson et al., 2014) and methotrexate resistance through amplification of dihydrofolate reductase encoded in ecDNA (Shoshani et al., 2021). Indeed, the failure of targeted therapeutics to deliver meaningful impact in glioblastomas, targeting oncogenic drivers such as EGFRvIII, may be explained by the high prevalence of ecDNA in this disease (Turner et al., 2017). Accumulation of ecDNA within localized hubs has also been shown to result in oncogene overexpression through enhancer-gene interactions (Hung et al., 2021). ecDNA offers a target for therapeutic intervention by attenuating extreme oncogene amplification, expression, and tumor heterogeneity. As a promising avenue, ecDNA hubs have been shown to be disrupted through BET protein inhibition (Hung et al., 2021).

Enhancing immune recognition of aneuploid cells may be a cancer cell extrinsic approach to exploiting CIN. One mechanism thought to be involved in this surveillance system *in vivo* is the cGAS-STING pathway, whose activation by cytosolic DNA from micronuclei rupture links CIN to metastasis (Bakhoum et al., 2018). Targeting this and other mechanisms of escape

from immune recognition of aneuploidy could offer new routes to forestall therapy resistance.

Undruggable targets and adaptation to targeted therapeutic pressure

One of the major challenges over the last 2 decades has been the targeting of clonal driver alterations, such as p53 and KRAS, traditionally considered undruggable (Cox et al., 2014). In 2013, Ostrem et al. (2013) reported on the development of small molecules that could irreversibly bind to KRAS G12C, providing confirmation that oncogenic mutations in KRAS could be specifically targeted. On May 28th, 2021, the US Food and Drug Administration (FDA) approved the KRAS G12C inhibitor sotorasib for use in adult patients with NSCLC. In the phase II CodeBreaK100 trial of 126 patients with advanced KRAS G12C mutant NSCLC previously treated with standard therapy, sotorasib led to an objective response in 37.1%, with a median duration of response of 11.1 months (Skoulidis et al., 2021); however, among 62 patients with CRC, the overall response rate was 9.7%, with median progression-free survival of 4.0 months (Fakih et al., 2022). This stark difference may be a consequence of the tissue of origin and microenvironmental context of the mutation and is a contributing factor to limitations of the genotype-matched targeted treatment approach (Middleton et al., 2021).

Tumors may display a rapid adaptive response or slower, acquired resistance to therapeutic pressures. An adaptive response usually occurs as a consequence of negative feedback and pathway redundancy in receptor tyrosine kinase signaling that results in parallel pathway activation or upstream reactivation of the targeted pathway. Acquired resistance mechanisms are achieved in several ways, including gatekeeper mutations, oncogene amplification, splice variants, or driver mutations that affect ATP-competitive tyrosine kinase inhibitor (TKI)-binding sites (Vasan et al., 2019). In many cases, acquired resistance can be heterogeneous; for example, in a longitudinal study of 59 patients with relapsed and/or refractory *FLT3* mutant acute myeloid leukemia (AML), multiple activating mutations in the RAS/mitogen-activated protein kinase (MAPK)-signaling pathway were most frequently observed, with polyclonal and diverse patterns of selection (McMahon et al., 2019).

Through comprehensive mapping of key resistance pathways, some interesting observations have been made regarding the complexity of this adaptive process, primarily that, despite the diverse and complex pathways of resistance found in tumor cells, these pathways often converge toward common downstream signaling pathways that are conserved in residual and resistant tumor cells. It may therefore be possible to utilize a combination therapy strategy that exploits this collateral sensitivity while mitigating systemic toxicity (Lin et al., 2020; Singleton et al., 2017; Wood, 2015).

As a response to therapeutic pressure, tumor cells can enter a drug-tolerant persister (DTP) state, a reversible epigenetic-driven phenotype switch whereby a subclonal population arrests or cycles slowly in the presence of a drug and a source of non-genetic heterogeneity (Vallette et al., 2019). Recently, it has been shown that a rare subpopulation of persister cells can proliferate under treatment. By developing a system to simultaneously track individual cell clonal origin, proliferative, and transcriptional states, Oren et al. (2021) showed that persister cells exhibited a higher expression of glutathione metabolism and an NRF2 signature. NRF2 is an oxidative-stress-induced transcription factor, and it is possible that the escape from senescence is linked to the ability of the cell to mitigate oxidative stress. Furthermore, the authors also demonstrated that cycling persister cells are dependent on a metabolic shift to fatty acid oxidation, highlighting a potential treatment strategy to target this metabolic constraint (Oren et al., 2021). Through combining CRC-patient-derived xenograft models with high-complexity lentiviral barcoding, RNA-seq, whole-exome sequencing, and mathematical modeling, Rehman et al. (2021) demonstrated that tumors that entered a DTP state retained clonal complexity following withdrawal of treatment. Furthermore, the authors demonstrated that a DTP state is phenotypically and transcriptionally similar to diapause, a period of developmental dormancy utilized by insects and mammalian embryos through adverse environmental conditions (Dhimolea et al., 2021; Rehman et al., 2021).

Mitigating acquired resistance may be enabled by taking advantage of the potential fitness cost following the evolution of targeted therapy resistance. It has been observed that mutant RAS clones that are resistant to anti-EGFR monoclonal antibodies diminish on withdrawal of treatment, leading to re-emergence of drug-sensitive clones. This phenomenon is being exploited in the CHRONOS trial, from which patients with metastatic CRC treated with anti-EGFR therapy are re-challenged following monitoring of *RAS*, *BRAF*, and *EGFR* mutational status through ctDNA (Sartore-Bianchi et al., 2021).

Immunogenicity and tumor microenvironment

The tumor microenvironment is constituted of immune cells, stroma, and vasculature and is a determinant of drug resistance in many cancer types. This is well demonstrated in the context of checkpoint inhibitors (CPIs), drugs that stimulate tumor immune responses through reactivation of tumor-antigen-specific T cells. In a meta-analysis of CPI response that utilized whole-exome sequencing and RNA-seq data, comprising studies from seven tumor types, including over 1,000 CPI-treated patients, clonal tumor mutational burden (TMB) and *CXCL9* expression were the strongest predictors of response (Litchfield et al., 2021). In this study, *CCND1* amplification was also associated with resistance. There are many other tumor-intrinsic mechanisms of CPI resistance described, such as *JAK1*/*JAK2* mutations that attenuate the expression of interferon-stimulated genes (Shin et al., 2017; Zaretsky et al., 2016) and activating mutations of RTK genes (point mutations and amplifications in *EGFR* and *ERBB2* and amplifications in *MET*, *FGFR1*, and *IGF1R*), which are implicated in the regulation of immune responses through the MAPK and phosphatidylinositol 3-kinase (PI3K)-AKT-mTOR pathways and are independent of TMB (Anagnostou et al., 2020). Furthermore, the immunosuppressive tumor environments have distinct phenotypes (immune excluded, immune desert, and inflamed) that are associated with differing responses to immunotherapy (Chen and Mellman, 2017).

The goal of remodeling the tumor microenvironment to improve tumor immunogenicity holds much promise. Transforming growth factor β (TGF-β) has a complex and diverse role in tumor physiology, including the initiation of epithelial-to-mesenchymal transition (EMT) in tumor cells and suppression of anti-tumor immunity through activation of cancer-associated

fibroblasts (Liu et al., 2021; Tauriello et al., 2018). Li et al. (2020a) demonstrated that selectively targeting TGF-β signaling in $CD4^+$ T cells may enhance tumor immunity, and as part of the IMvigor210 trial, Mariathasan et al. (2018) demonstrated that TGF-β activation in fibroblasts was significantly associated with non-response in immune-excluded tumors, with combined blockade of TGF-β and PD-L1 resulting in a significant reduction of tumor burden in an EMT6 mouse mammary carcinoma model. This approach was the basis of the INTR@PID Lung 037 trial, which compared bintrafusp alfa, a bifunctional fusion protein targeting both TGF-β and PD-L1, with pembrolizumab in PD-L1-expressing advanced NSCLC (NCT03631706). The trial failed to meet its primary endpoint, with similarly disappointing results in the phase II INTR@PID BTC 055 trial involving patients with locally advanced or metastatic biliary tract cancer (NCT04066491). Evaluation of bintrafusp alfa in patients with HMGA2-expressing triple-negative breast cancer (NCT04489940) and advanced, unresectable cervical cancer (NCT04246489) is ongoing.

An intriguing avenue to attempt to improve immune checkpoint inhibition response is through the generation of *de novo* immunogenic mutations. This approach is being assessed through the ongoing ARETHUSA trial, whereby mismatch-repair-proficient, RAS mutant patients with metastatic CRC and O6-methylguanine-DNA methyltransferase (MGMT) deficiency (assessed through promoter methylation analysis and immunohistochemistry) are treated with temozolomide to increase TMB. In those patients with TMB over 20 mutations per megabase, the patients are subsequently randomized to the anti-PD-1 inhibitor pembrolizumab (Siena et al., 2019). The first stage of the phase 2 TONIC trial randomized 67 patients with metastatic triple-negative breast cancer to receive another PD-1 inhibitor, nivolumab, with either no induction, irradiation (3 × 8 Gy), cyclophosphamide, cisplatin, or doxorubicin preceding this (Voorwerk et al., 2019). The overall response rate was greatest in patients who had received cisplatin and doxorubicin at 23% and 35%, respectively. Moreover, an upregulation of immune-related genes involved in PD-1 and PD-L1 and cytotoxic T cell signaling was reported in samples post-cisplatin and doxorubicin induction (Voorwerk et al., 2019).

There has been recent success of adoptive T cell therapies, both through expansion of tumor-infiltrating lymphocytes (TILs) and chimeric antigen receptor T (CAR-T) cells (Rosenberg and Restifo, 2015). Parallel developments in the field of adoptive T cell therapies aim to target multiple clonal neoantigens in an effort to prolong efficacy and limit adoptive T cell therapy resistance (McGranahan et al., 2016). In the CAR-T field, the multicenter ELIANA study of 75 patients with pediatric relapsed or refractory acute lymphoblastic leukemia (ALL) demonstrated the efficacy of the CD19 CAR-T cell tisagenlecleucel, with 81% remission rate at 3 months and overall survival of 76% at 12 months, leading to the European Medicines Agency (EMA) and FDA approval in 2017 (Maude et al., 2018). CAR-T therapy holds great promise for multiple cancer types, with recent FDA approvals in DLBCL (JULIET trial), multiple myeloma (Teoh and Chng, 2021), and mantle cell lymphoma (Wang et al., 2020) and renewed focus to translate this success to solid tumors, including prostate cancer (Bagley and O'Rourke, 2020; Wolf et al., 2021). Disease relapse in this treatment setting is broadly divided between tumor-intrinsic mechanisms, which include antigenic escape, and the failure of CAR-T-cell-mediated surveillance, usually due to loss of persistence (Shah and Fry, 2019). CD19 antigenic loss was reported in 25% of patients in the ELIANA study and has been well described in the context of other studies (Majzner and Mackall, 2018). One mechanism of antigenic loss that has been well described is the positive selection of splice variants that lack the exons encoding either the extracellular epitope or the transmembrane domain of CD19 (Sotillo et al., 2015). Antigenic loss may be attenuated through targeting of multiple antigens, such as CD19 and CD22 in refractory B cell ALL (B-ALL) (Cordoba et al., 2021). Strategies to mitigate loss of CAR-T persistence include changes to the co-stimulatory domain and immunoreceptor tyrosine-based activation motif, with the overall goal of optimizing activation without inducing exhaustion (Berger and Maus, 2021). These modifications are incorporated in next-generation CAR-T cells (Tokarew et al., 2019). Novel classes, such as synthetic enzyme-armed killer (SEAKER) cells, CAR-T cells that are engineered to activate prodrugs at tumor sites, indicate further exciting developments in this field (Gardner et al., 2021).

As sequencing continues to become more affordable, trial endpoints will more commonly incorporate high-throughput DNA sequencing and RNA-seq data and tumor microenvironment analysis to decipher the underlying causes of drug resistance and treatment failure in each patient. The field must continue to adapt to clinical areas of need, describing resistance in agents that are commonly used in the clinic and adapting treatment strategies accordingly. Examples of this include the development of osimertinib, developed following the identification of the T790M mutation in EGFR mutant NSCLC (Kobayashi et al., 2005), which attenuates the binding of first- and second-generation TKIs to the ATP binding site in EGFR (Cross et al., 2014). Through large, well-designed, clinically annotated, longitudinal studies, it will become possible to capture these diverse mechanisms in the clinic.

CANCER EVOLUTION IN METASTASIS

Metastasis is a complex, multi-stage process whereby cancer cells disseminate from a primary tumor to distant anatomical sites. Metastatic disease remains incurable for the majority of solid tumors, accounting for >80%–90% of cancer-related deaths (Lambert et al., 2017; Massagué and Obenauf, 2016). As DNA sequencing of metastatic samples has become readily available (Nguyen et al., 2022; Priestley et al., 2019), analyses of recurrent genomic patterns in large metastatic cohorts have identified putative metastatic drivers (Priestley et al., 2019; Turajlic and Swanton, 2016). For example, the loss of chromosome 9p was reported as a highly selected event driving metastasis from the analysis of 575 primary and 335 metastatic biopsies across 100 patients with metastatic clear-cell renal cell carcinoma in the Renal TRACERx program (Turajlic et al., 2018). Other examples include *MYC* amplification in lung and prostate adenocarcinoma as well as other cancer types (Nguyen et al., 2022; Shih et al., 2020). Loss of *CDKN2A/B* may also play a role in lung, pancreatic, and esophageal adenocarcinoma, among others (Nguyen et al., 2022; Shih et al., 2020). Ongoing studies are investigating the development of novel targeted drugs for some of these genes, such as MAX-binding inhibitors for *MYC*

amplification (Duffy et al., 2021) and *PRMT5* inhibitors for *CDKN2A*-deleted tumors (Mavrakis et al., 2016).

A detailed understanding of the mechanisms underlying the process of metastatic dissemination would further support the development of targeted therapeutic approaches (Massagué and Obenauf, 2016; Turajlic and Swanton, 2016), and recent studies have focused on investigating which cancer cell clones are driving the metastatic process within heterogeneous primary tumors. In early metastatic studies (Poste and Fidler, 1980; Fidler, 2003; Talmadge and Fidler, 2010; Liu et al., 2009), the dominant model of metastatic spread was a monoclonal model, where a single tumor clone acquires the traits required to metastasize and all metastatic cancer cells descend from it. However, recent studies have provided evidence that different metastases originate from distinct tumor subclones and that even a single metastasis might originate from the migration of distinct tumor clones from the primary tumor or from other metastases (Comen et al., 2011; Gundem et al., 2015; El-Kebir et al., 2018).

The polyclonal nature of many tumors might result from necessary cooperative interaction between different clones to support tumor growth through clone-clone cooperative interactions. Technological and methodological advancements have allowed recent studies to demonstrate inter-clonal interactions in both *in silico* or mouse models (Cleary et al., 2014; Marusyk et al., 2014; McFadden et al., 2014) and in human cancer cell lines (Janiszewska et al., 2019; Ombrato and Malanchi, 2019). The importance of stromal cell recruitment to the tumor microenvironment has been demonstrated in the past few years (Hanahan and Coussens, 2012; Hanahan and Weinberg, 2011), but the role of distinct cooperating tumor clones still remains unclear.

Although polyclonality complicates the understanding of metastatic mechanisms (El-Kebir et al., 2018), it also yields the opportunity to target tumor clones with different roles in the metastatic process and to disrupt interactions between subclones in the same tumor. Single-cell sequencing technologies are demonstrating the possibility to identify distinct tumor subclones independent of their prevalence in human tumors, paving the way to functionally characterize their role in the metastatic process. For example, Chan et al. (2021) used single-cell sequencing technologies to identify recurrent small subclones with PLCG2 overexpression from 21 small cell lung cancer patients and suggested that such small subclones might have a critical role in supporting metastatic progression in these cancers, possibly through an interaction with monocyte and macrophage populations that facilitate an immunosuppressive tumor microenvironment. Quinn et al. (2021) have recently used these single-cell technologies to develop a Cas9-based lineage tracer to track the evolutionary history and routes of dissemination of single metastatic cancer cells in cancer xenografts, revealing different patterns of dissemination. Such studies provide a framework that can be used to functionally validate the cooperative interactions between distinct tumor clones and to investigate the underlying cellular mechanisms that may lead to future treatment approaches.

PRACTICAL CHALLENGES TO WIDER APPLICATION

There are a number of practical challenges to the widespread adoption of molecular profiling of cancer patients in translational research and clinical practice. In translational research, large-scale prospective and longitudinal studies of cancer patients that incorporate multiple sampling with genomic analysis and detailed clinical histories, imaging, and pathology analysis are rare. Comprehensive tumor sampling facilitates the detailed assessment of intratumor heterogeneity, and this often requires the sampling of multiple regions in a surgically resected specimen. Furthermore, sampling at relapse or recurrence is not always possible, especially in the context of the invasiveness of the procedure and underlying comorbidities and potential benefit of the procedure to the patient. The use of fixed formalin paraffin embedded (FFPE) tissue samples, widely used in biobanks, often limits the quality of RNA and DNA that can be extracted from the specimens. In addition, a robust research infrastructure involving collaboration with hospital departments, clinical trial units, and research laboratories is essential in curating such data for research (Bailey et al., 2021). Tackling these hurdles has provided rationale behind large multi-region and longitudinal studies, such as the Glioma Longitudinal Analysis (GLASS) consortium studies (GLASS Consortium, 2018), TRACERx (ClinicalTrials.gov Identifier: NCT01888601 & NCT03226886) (Jamal-Hanjani et al., 2017) and the UK national research autopsy study, PEACE (Posthumous Evaluation of Advanced Cancer Environment; ClinicalTrials.gov Identifier: NCT03004755).

Perhaps one of the biggest challenges lies at the translational interface of basic and clinical research. From the perspective of drug resistance, the importance of the scientific question is linked to what is observed in the clinic and resources should be directed as such. Infrastructure aimed at enhancing the dialogue between basic scientists and clinicians, including specific educational training programs, should continue to develop.

In clinical practice, sequencing costs remain prohibitive to its broader uptake. The National Human Genome Research Institute (NHGRI) has estimated that the current cost of sequencing a whole human genome has plateaued at approximately $1,000, and this does not consider the cost of additional resources, such as consumables, staff costs, and bioinformatic analysis (Schwarz et al., 2015). Beyond suitable infrastructure and costs, the challenges to obtaining samples of sufficient quantity and quality for analysis are relevant to both research and routine clinical practice.

CONCLUSION

The advances in genomic technologies have facilitated detailed genomic profiling of tumor types that have led to extensive categorization of cancer-related genes; however, the early promise of precision-based and personalized treatment strategies have not been fully realized in the clinical context. There are many reasons for this, including the incomplete categorization of SVs, epigenomic and transcriptional driver alterations, incomplete understanding of the interplay between the tumor and the microenvironment, the attenuation of anti-tumor immunity during disease progression, and an inability to fully prevent intrinsic, adaptive, and acquired drug-resistance processes. A more complete understanding of these complex processes will be key to translational success, and this will be achieved through extensive collaborative efforts between research groups and clinical teams.

We have the tools to progress our understanding of aging, somatic evolution, and the interface between clonal expansion

and cancer initiation; however, there may be limitations to translating these discoveries to further the field of cancer interception. There have been exciting developments in the early detection and minimal residual disease fields, and as advances are made to improve ctDNA limits of detection, the combination of cancers detected at an earlier stage together with the refinement of neoadjuvant and adjuvant treatment strategies will improve outcomes for cancer patients. We may see further implementation of targeted chemoprevention, vaccines for prevention, and refinement in quantifying germline risk for better screening programs.

Despite this, the global burden of cancer will continue to increase. This is in part due to an aging population; widening disparities between countries, socio-economic groups, and ethnicities; and exposure to environmental carcinogens, all of which will be further driven by the impact of climate change. The rising cost of drugs will also mean many patients will not benefit from cutting-edge discoveries discussed in this review. This burden can be mitigated through robust cancer prevention strategies, which will benefit from implementation of policies that address global inequalities in standard of living and access to affordable healthcare together with meaningful efforts to address climate change.

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DECLARATION OF INTERESTS

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