The translational challenges of precision oncology

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Abstract

The translational challenges in the field of precision oncology are in part related to the incredible 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 in the future. 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 socio-economic status. The reasons for the increase in incidence include both a growing and ageing population. However, for multiple tumour types the age-specific risk is also increasing (Smittenaar et al., 2016). Environmental exposures are linked to the increasing age-specific risk of many tumour 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 tumour. 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 tumour microenvironment in governing tumour 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 in the future. 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 to 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 future 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 findings, such as the heritability of breast cancer reported by Pierre Paul Broca (Broca P., 1866), the observation of aberrant mitoses by David Von Hansemann (Hansemann, David, 1890), and the finding by Theodor Boveri that abnormal chromosomal segregation was sufficient to cause malignant proliferation (Boveri, 1914). 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 sequencing (Bailey et al., 2018; McLendon et al., 2008a; Parsons et al., 2008, 2008; Sjöblom et al., 2006) revealed that the coding regions of the tumour genome harboured from tens of point mutations in acute myeloid leukaemias 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 tumour 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 9423 exomes from 33 tumour 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 tumours across 66 tumour types uncovered 568 mutational drivers (Martínez-Jiménez et al., 2020). Other key studies have focussed on the characterisation 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 tumourigenesis. Alterations through these mechanisms include focal amplifications of *EGFR* in glioblastoma multiforme (GBM) (McLendon et al., 2008b), *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 these events in tumorigenesis is far less characterised 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 tumours (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 tumours within the Pan Cancer Analyses of Whole Genomes consortium (PCAWG) (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 characterise 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 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 in NSCLC 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 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 (Middleton et al., 2021).

Through progress in this field 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 have 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 tumour 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/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 are other complicating factors that demonstrate that the original concept of precision oncology was not the panacea that it initially promised to be.

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 ageing-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 utilised 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 have been detailed across different tissues, including skin (Martincorena et al., 2015), colon (Lee-Six et al., 2019), oesophagus (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 under positive selection in non-cancerous tissue, including *NOTCH1* (skin, bronchus and oesophagus), *TP53* (oesophagus, bronchus) and *PIK3CA* (endometrium, oesophagus) (Kakiuchi and Ogawa, 2021). Mutations found in RNA-seq data from healthy individuals within the GTEX consortium also revealed clonal expansions in different tissues (García-Nieto et al., 2019; Yizhak et al., 2019). Somewhat different to mutations, DNA copy number aberrations were observed in less than 10% (37/389) of 389 samples across 29 different histologies (Moore et al., 2021). Interestingly, oesophageal and cardiac tissues harbour more copy number alterations than other organ sites (R. 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 oesophageal

epithelium (~66%) but less frequently in oesophageal 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 to 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 tumour 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 to 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).

Unravelling mechanisms that drive the clonal expansion of normal tissue towards 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 tumour 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 *BRAF* V600E is affected by the location and 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, Signature 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, 17/20 known suspected carcinogens in mice increased tumorigenesis but did not increase mutational burden or generate a specific mutational process (Riva et al., 2020). Furthermore, the Mutograph project revealed no distinct mutational patterns in oesophageal 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 which 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, oesophageal 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 tumour microenvironment, permitting clonal expansions and transformation.

Immune surveillance, ageing and senescence

Tumour 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 tumour-intrinsic immune escape events including loss of *HLA* alleles and mediated by the tumour microenvironment (McGranahan and Swanton, 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 tumour 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 ageing, increasing from 25 cases per 100,000 in those less than 20 years old to more than 1000 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 ageing, 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 co-incidence 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 ageing 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 neighbouring cells (Coppé et al., 2010; Rodier et al., 2009). This sustained and systemic low-grade chronic inflammation is one of the hallmarks of ageing and is termed 'inflammaging' (López-Otín et al., 2013). The number of senescent cells increases exponentially with ageing (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 $CD8^+$ activity through the recruitment of myeloid-derived suppressor cells and Tregs 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 cell function 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 tumour 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 tumour types using the MSK-IMPACT panel (which targets 341 cancer-related genes) revealed that ~7.8% patients harboured 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 MHC-I and MHC-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 *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 tumour characterisation, 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 tumour 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 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 labelled 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 LDL cholesterol synthesis used to reduce the incidence of cardiovascular disease. This has also been utilised 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 of 65% and 49% after treatment with exemestane and anastrozole, respectively. However, adverse effects associated with exposure to tamoxifen (endometrial cancers, 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 cyclooxygenase (COX) 1 and 2 inhibitors, such as aspirin, lowers the risk of distant metastasis and increases survival in CRC (Liao et al., 2012; Rothwell et al., 2012). Furthermore, from a pooled analysis of 2 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 signalling 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. Vaccines based on viral antigens from the human papillomavirus (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, tumour-specific neoantigens are the targets of effective tumour-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 HLA class I typing from individual patients. There are a number of ongoing trials of personalised neoantigen vaccines in patients with established solid tumours (NCT03633110, NCT03289962, 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 tumour 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 oesophagus (BE), a gastro-oesophageal reflux-related precursor lesion to oesophageal adenocarcinoma (EAC) characterised by metaplasia of the epithelium (Spechler, 2013). Patients with BE 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 amongst 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 Barrett’s oesophagus detection amongst those randomised 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 premalignant 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 characterisation has revealed differences between premalignant regions that spontaneously regress and those that progress to invasive disease over time.

Progressive lesions harboured 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 premalignant lesions and development of methods to detect them. In cases such as cervical adenocarcinoma in situ (AIS) and Barrett's oesophagus, 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 Barrett's (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 premalignant 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 programmes that utilise 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 maximise 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 one-year net survival of patients diagnosed at stage 1 was 97.7% compared with 43.9% at stage 4 between 2013-2017. For lung cancer one-year net survival at stage 1 was 87.7% and at stage 4 it was 19.3%, and 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 programmes 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 CT screening at baseline, 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 randomised 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 personalised 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 circulating tumour DNA (ctDNA). ctDNA is the tumour specific fraction of cell free DNA (cfDNA); extracellular DNA that is released into the plasma. ctDNA has great potential as a minimally invasive tumour biomarker, and there are numerous studies demonstrating that amount of ctDNA detected in the plasma correlates with tumour burden, metabolism and rate of proliferation (Abbosh et al., 2017a; 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 tumour specific mutations with protein biomarkers. In 2018, Cohen et al used CancerSEEK to study 1005 patients with 8 tumour types of stages I-III, with the assay detecting mutations in 1933 distinct genomic positions and eight tumour specific 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) (Cohen et al., 2018). 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 PET-CT (where positive), to confirm and localise the site of disease (Lennon et al., 2020). Of 96 incident cancers, 26 were picked up through blood testing from which fifteen 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 are 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; amongst 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 amongst 6689 participants (including 2482 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 stages 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 randomising 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 analyses the product of differential enzymatic fragmentation in tumour and non-tumour 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 future of ctDNA assays in early detection will be determined through our understanding of cell-free DNA (cfDNA) and ctDNA kinetics, with the hope that patients with tumours that are ‘born to be bad’ can be identified and neoadjuvant treatment tailored accordingly.

Adjuvant Therapy and Minimal residual disease monitoring (MRD)

The application of next-generation sequencing approaches to minimal residual disease monitoring (MRD) has led to 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 improves 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., 2017b). The 'personalised' approach of utilising tumour-specific mutations, developed through 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). In 94 patients diagnosed with multiple tumour types as part of the INSPIRE trial, baseline ctDNA concentration correlated with clinical response, progression-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). 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).

Tracking tumour mutations in the context of low tumour burden and at low allele frequencies presents a challenge. Zviran et al adopted an alternative approach (MRDetect) to targeted deep sequencing through whole-genome sequencing of cfDNA (Zviran et al., 2020). MRDetect utilises the cumulative signal of thousands of tumour mutations as priors to enhance the detection threshold of variants at both low allele frequencies and ctDNA fraction (Zviran et al., 2020). By utilising phased variants derived from whole-genome sequenced tumour samples (PhasED-seq), Kurtz et al 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 (Kurtz et al., 2021).

Beyond early detection and MRD monitoring, the utility of ctDNA in selecting patients for mutation directed therapy was assessed in the plasmaMATCH trial, a multicentre phase 2a platform trial of 1051 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 tumour biopsies at 98% (95% 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 we expect to see this rolled out for multiple tumour types in the near 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 intratumour heterogeneity, adaptive responses to targeted therapeutic pressures, cancer immunogenicity and the impact of the tumour 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 tumour suppressor proteins. Here we explore contributions to four of those key determinants (**figure 3**), and address the broad areas for future research.

Intratumour heterogeneity

As cancers evolve somatic mutations accumulate through temporally and spatially distinct mutational processes. While this in itself is enough to create genetic diversity, a subset of these mutations will confer fitness advantage and drive clonal expansions of cancer cells. These clonal expansions contribute to the genetically distinct subclonal populations of cancer cells that underlie the intratumour heterogeneity (ITH) observed across cancer types through bulk multi-region sequencing and single-cell sequencing approaches (Gerlinger et al., 2012; Marusyk et al., 2020; McGranahan and Swanton, 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 tumour 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 preexisting 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 a genetic screen to silence mitotic kinesin (Marquis et al., 2021) using sequencing data from the TCGA, essentiality data from cancer cell lines (Quinton et al., 2021) genomics data from 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 structural variants that often harbour 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 remodelling (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 to 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 localised 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 tumour 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 two 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 reported on the development of small molecules that could irreversibly bind to KRAS G12C, providing confirmation that oncogenic mutations could be specifically targeted (Ostrem et al., 2013). On May 28th 2021, the 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, sotarisib led to an objective response in 37.1%, with a median duration of response of 11.1 months (Skoulidis et al., 2021), however amongst 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).

Tumours 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 signalling 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 TKI binding sites (Vasan et al., 2019). In some cases, acquired resistance can be heterogeneous; for example, in a longitudinal study of 59 patients with relapsed and/or refractory *FLT3* mutant AML, multiple activating mutations in the RAS/MAPK signalling 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 tumour cells, these pathways often converge towards common downstream signalling pathways that are conserved in residual and resistant tumour cells. It may therefore be possible to utilise a combination therapy strategy that exploits this collateral sensitivity whilst mitigating systemic toxicity (Lin et al., 2020; Singleton et al., 2017; Wood, 2015).

As a response to therapeutic pressure, tumour 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 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 (Oren et al., 2021). 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 modelling, Rehman et al demonstrated that tumours that entered a DTP state retained clonal complexity following withdrawal of treatment. (Rehman et al., 2021). Furthermore, the authors demonstrated that a DTP state is phenotypically and transcriptionally similar to diapause, a period of developmental dormancy utilised 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 tumour microenvironment

The tumour 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 (CPI), drugs that stimulate tumour immune responses through reactivation of tumour antigen-specific T cells. In a meta-analysis of CPI response that utilised whole-exome sequencing and RNA-seq data, comprising studies from seven tumour types including over 1000 CPI treated patients, clonal tumour 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 tumour 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 mitogen-activated protein kinase and PI3K-AKT-mTOR pathways and are independent of TMB (Anagnostou et al., 2020). Furthermore, the immunosuppressive tumour 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 remodelling the tumour microenvironment to improve tumour immunogenicity holds much promise. TGF-β has a complex and diverse role in tumour physiology, including the initiation of epithelial-to-mesenchymal transition (EMT) in tumour cells and suppression of anti-tumour immunity through activation of cancer-associated fibroblasts (Liu et al., 2021; Tauriello et al., 2018). Li et al demonstrated that selectively targeting TGF-β signalling in $CD4^+$ T cells may enhance tumour immunity (Li et al., 2020a), and as part of the IMvigor210 trial, Mariathasan et al demonstrated that TGF-β activation in fibroblasts was significantly associated with non-response in immune-excluded tumours, with combined blockade of TGF-β and PDL-1 resulting in a significant reduction of tumour burden in an EMT6 mouse mammary carcinoma model (Mariathasan et al., 2018). This approach was the basis of the INTR@PID Lung 037 trial which compared bintrafusp alpha, a bifunctional fusion

protein targeting both TGF-β and PDL-1, to 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 alpha 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 randomised to the anti-PD-1 inhibitor pembrolizumab (Siena et al., 2019). The first stage of the phase 2 TONIC trial randomised 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/PD-L1 and cytotoxic T cell signalling 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 tumour 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 multicentre ELIANA study of 75 patients with paediatric relapsed or refractory acute lymphoblastic leukaemia (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 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), mantle cell lymphoma (Wang et al., 2020), and renewed focus to translate this success to solid tumours including prostate cancer (Bagley and O'Rourke, 2020, Wolf 2021). Disease relapse in this treatment setting is broadly divided between tumour intrinsic mechanisms, which include antigenic escape, and the failure of CAR-T cell-mediated surveillance, usually due to loss

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 acute lymphoblastic leukemia (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 optimising 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 SEAKER (synthetic enzyme-armed killer) cells, CAR-T cells that are engineered to activate prodrugs at tumour 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/RNA sequencing data and tumour 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 tumour to distant anatomical sites. Metastatic disease remains incurable for the majority of solid tumours, 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., 2021; 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 tumours (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 tumours. 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 tumour clone acquires the traits required to metastasise and all metastatic cancer cells descend from it. However, recent studies have provided evidence that different metastases originate from distinct tumour subclones and that even a single metastasis might originate from the migration of distinct tumour clones from the primary tumour or from other metastases (Comen et al., 2011; Gundem et al., 2015; El-Kebir et al., 2018).

The polyclonal nature of many tumours might result from necessary cooperative interaction between different clones to support tumour 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 tumour microenvironment has been demonstrated in the past few years (Hanahan and Coussens, 2012; Hanahan and Weinberg, 2011), but the role of distinct cooperating tumour clones still remains unclear.

Although polyclonality complicates the understanding of metastatic mechanisms (El-Kebir et al., 2018), it also yields the opportunity to target tumour clones with different roles in the metastatic process and to disrupt interactions between subclones in the same tumour. Single-cell sequencing technologies are demonstrating the possibility to identify distinct tumour subclones independent of their prevalence in human tumours, paving the way to functionally characterise their role in the metastatic process. For example, Chan et al. 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/macrophage populations that facilitate an immunosuppressive tumour microenvironment (Chan et al., 2021). Quinn et al. 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 (Quinn et al., 2021). Such studies provide a framework that can be used to functionally validate the cooperative interactions between distinct tumour 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 tumour sampling facilitates the detailed assessment of intratumour 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 GLASS (GLASS Consortium, 2018), TRACERx and PEACE (Jamal-Hanjani et al., 2017).

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 programmes, 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 $1000, 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 is relevant to both research and routine clinical practice.

Conclusion

The advances in genomic technologies have facilitated detailed genomic profiling of tumour types that have led to extensive categorisation of cancer-related genes, however the early promise of precision-based and personalised treatment strategies have not been fully realised in the clinical context. There are many reasons for this including the incomplete categorisation of structural variants, epigenomic and transcriptional driver alterations, incomplete understanding of the interplay between the tumour and the microenvironment, the attenuation of anti-tumour 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 ageing, 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 programmes.

Despite this, the global burden of cancer will continue to increase. This is in part due to an ageing 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, access to affordable healthcare together with meaningful efforts to address climate change.

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Declaration of Interests

C.S. acknowledges grant support from Pfizer, AstraZeneca, Bristol Myers Squibb, Roche-Ventana, Boehringer-Ingelheim, Invitae (previously Archer Dx Inc) - collaboration in minimal residual disease sequencing technologies, and Ono Pharmaceutical, and is an AstraZeneca Advisory Board member and Chief Investigator for the AZ MeRmaiD1 and 2 clinical trials. CS is also chief investigator of the NHS Galleri trial and has consulted for Amgen, Pfizer, Novartis, GlaxoSmithKline, MSD, Bristol Myers Squibb, Celgene, AstraZeneca, Illumina, Genentech, Roche-Ventana, GRAIL, Medicxi, Metabomed, Bicycle Therapeutics, Roche Innovation Centre Shanghai, and the Sarah Cannon Research Institute, C.S. had stock options in Apogen Biotechnologies and GRAIL until June 2021, has stock options in Epic Bioscience, and has stock options and is co-founder of Achilles Therapeutics. M.J-H. has consulted, and is a member of the Scientific Advisory Board and Steering Committee, for Achilles Therapeutics, has received speaker honoraria from Astex Pharmaceuticals and holds a patent PCT/US2017/028013 relating to methods for lung cancer detection.

Patents: C.S. holds European patents relating to assay technology to detect tumour recurrence (PCT/GB2017/053289); to targeting neoantigens (PCT/EP2016/059401), identifying patent response to immune checkpoint blockade (PCT/EP2016/071471), determining HLA LOH (PCT/GB2018/052004), predicting survival rates of patients with cancer (PCT/GB2020/050221), identifying patients who respond to cancer treatment (PCT/GB2018/051912), a US patent relating to detecting tumour mutations (PCT/US2017/28013) and both a European and US patent related to identifying insertion/deletion mutation targets (PCT/GB2018/051892).

The other authors do not declare any conflict of interests.

Figure Legends

Figure 1. Future directions in Cancer Initiation research

Clonal expansion in healthy tissues. The relationship between somatic evolution and tumorigenesis, from mutational landscape to selection and order of events.
Germline variation. Paired germline and tumor specific studies to understand the impact of germline variation on cancer initiation.

Tumour immunity and the microenvironment. The immune surveillance of somatic clones and its relationship with clone size, evolutionary trajectory and driver landscape.

Ageing and Senescence. The relationship between ageing, senescence, somatic evolution and tumorigenesis.

Environmental carcinogenesis. Understanding the processes of mutagenic and non-mutagenic environmental carcinogenesis and the effect of chronic inflammation on cancer initiation.

Cancer driver events beyond coding regions. Mapping non-coding driver events across tumour types and quantifying non-genomic methods of selection.

Figure 2. Key areas for translation in Cancer Prevention and Early Detection

Germline risk. Risk stratification of individuals who harbour germline variants that increase susceptibility to cancer.

Environmental exposures. Reduction of environmental exposures, including mitigating the effects of climate change.

Vaccination. Identification of tumour specific neoantigens or common oncogenic mutations as vaccine targets.

Chemoprevention. The use of preventative medication in high-risk individuals to reduce cancer incidence.

Interception. Methods to better characterise and detect pre-malignant tissue to determine risk of malignancy and further development of intervention strategies.

National screening. Adoption of enhanced screening protocols for high-risk individuals.

ctDNA screening. The use of ctDNA to detect early-stage malignancies and the development of methods to detect low burden disease.

Figure 3. Selected key areas in the drug resistance field

Intratumour heterogeneity. Genetic and non-genetic intratumour 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 tumour cells offer routes to forestall drug resistance.

Immunogenicity and tumour microenvironment. Stimulating tumour immune responses through checkpoint inhibition (CPI) and adoptive T cell therapies (including CAR-T cells) have

delivered success in attenuating tumour progression. Mechanisms of CPI and CAR-T resistance have been well characterised, from which strategies to remodel the tumour microenvironment, prevent antigenic loss and optimise T cell function will continue to develop.

Undruggable targets. The success of targeting clonal driver alterations such as KRAS G12C may herald the development of further therapies towards targets previously considered 'undruggable'.

Adaptation to targeted therapeutic pressure. 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 the fact that resistance pathways often converge on conserved downstream signalling pathways. **Drug tolerant persister cells** enter a reversible epigenetic driven phenotype that arrest or proliferate 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. Understanding the interplay between these mechanisms will lead to further approaches to tackling targeted therapy resistance.

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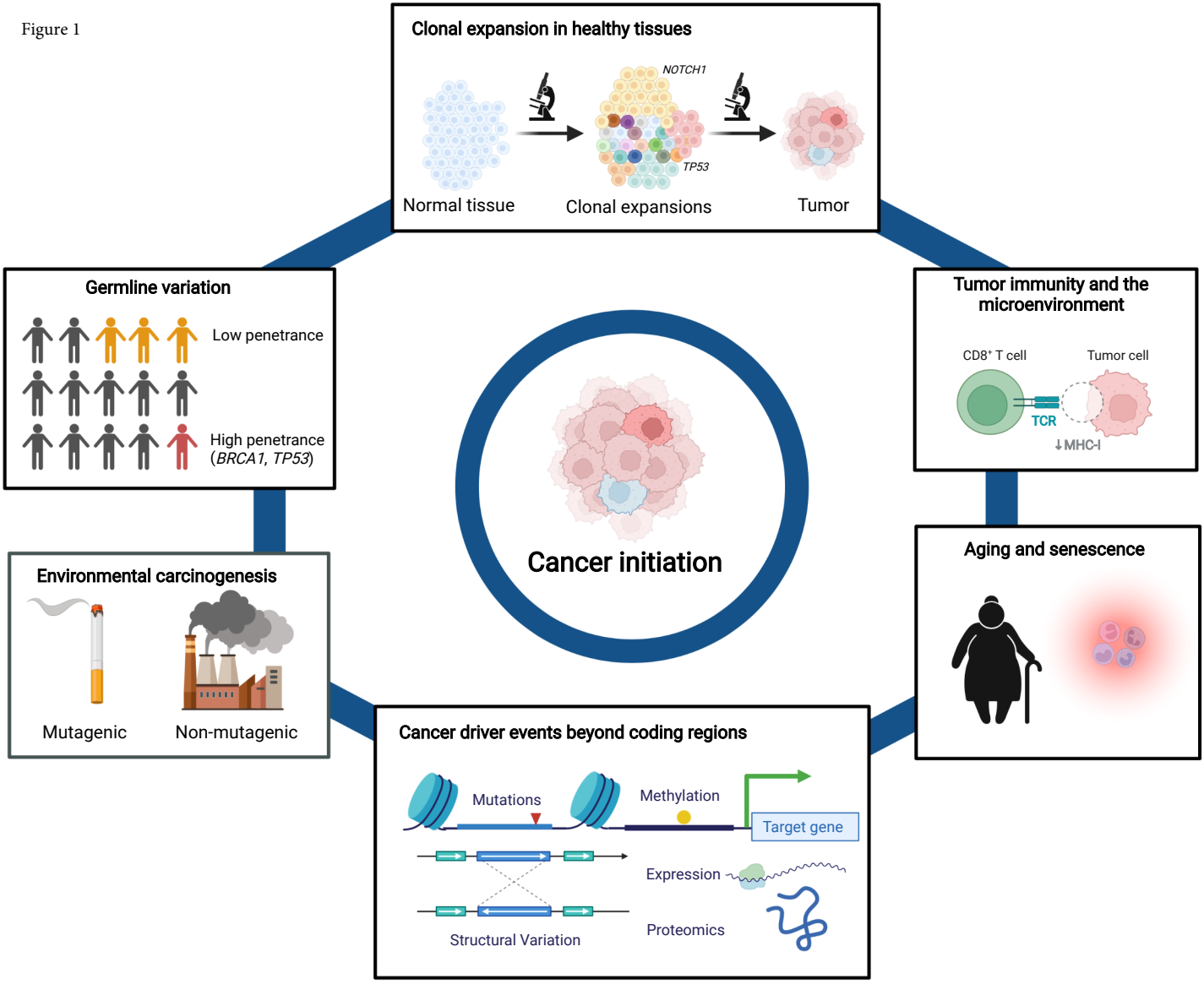
Figure 1


Clonal expansion in healthy tissues
NOTCH1
TP53
Normal tissue
Clonal expansions
Tumor
Germline variation
Low penetrance
High penetrance
(BRCA1, TP53)
Tumor immunity and the microenvironment
CD8+ T cell
Tumor cell
TCR
↓MHC-I
Cancer initiation
Environmental carcinogenesis
Mutagenic
Non-mutagenic
Aging and senescence
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Mutations
Methylation
Target gene
Expression
Proteomics
Structural Variation

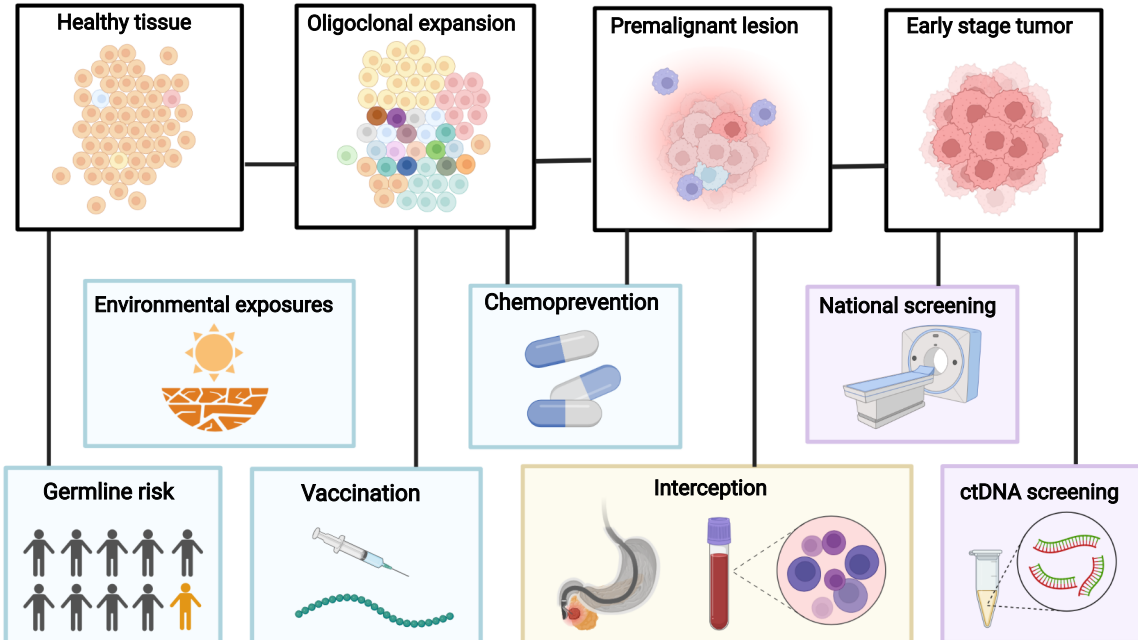
Healthy tissue
Oligoclonal expansion
Premalignant lesion
Early stage tumor
Environmental exposures
Chemoprevention
National screening
Germline risk
Vaccination
Interception
ctDNA screening

Figure 2

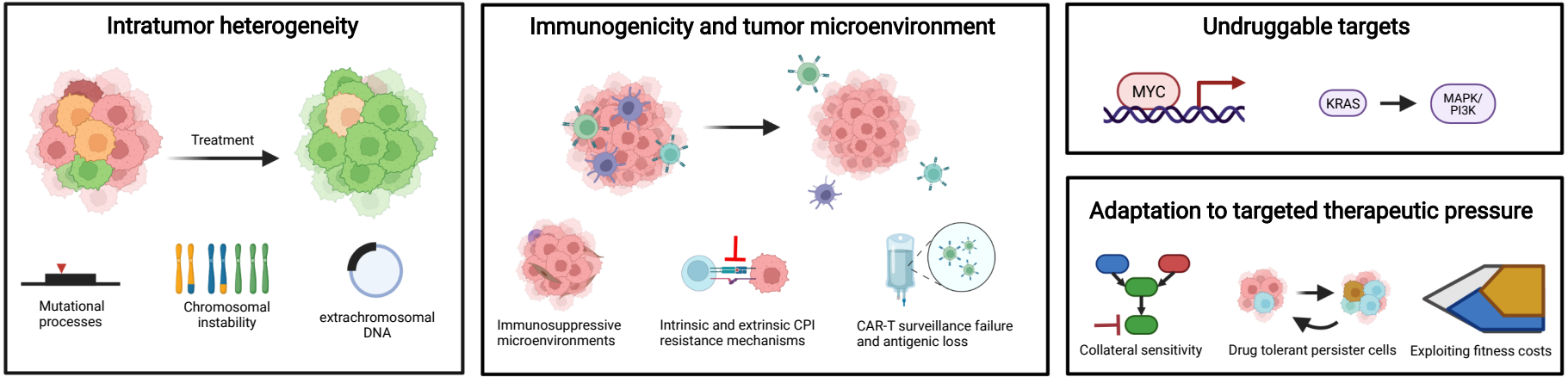
Intratumor heterogeneity
Treatment
Mutational processes
Chromosomal instability
extrachromosomal DNA
Immunogenicity and tumor microenvironment
Immunosuppressive microenvironments
Intrinsic and extrinsic CPI resistance mechanisms
CAR-T surveillance failure and antigenic loss
Undruggable targets
MYC
KRAS
MAPK/ PI3K
Adaptation to targeted therapeutic pressure
Collateral sensitivity
Drug tolerant persister cells
Exploiting fitness costs

Figure 3