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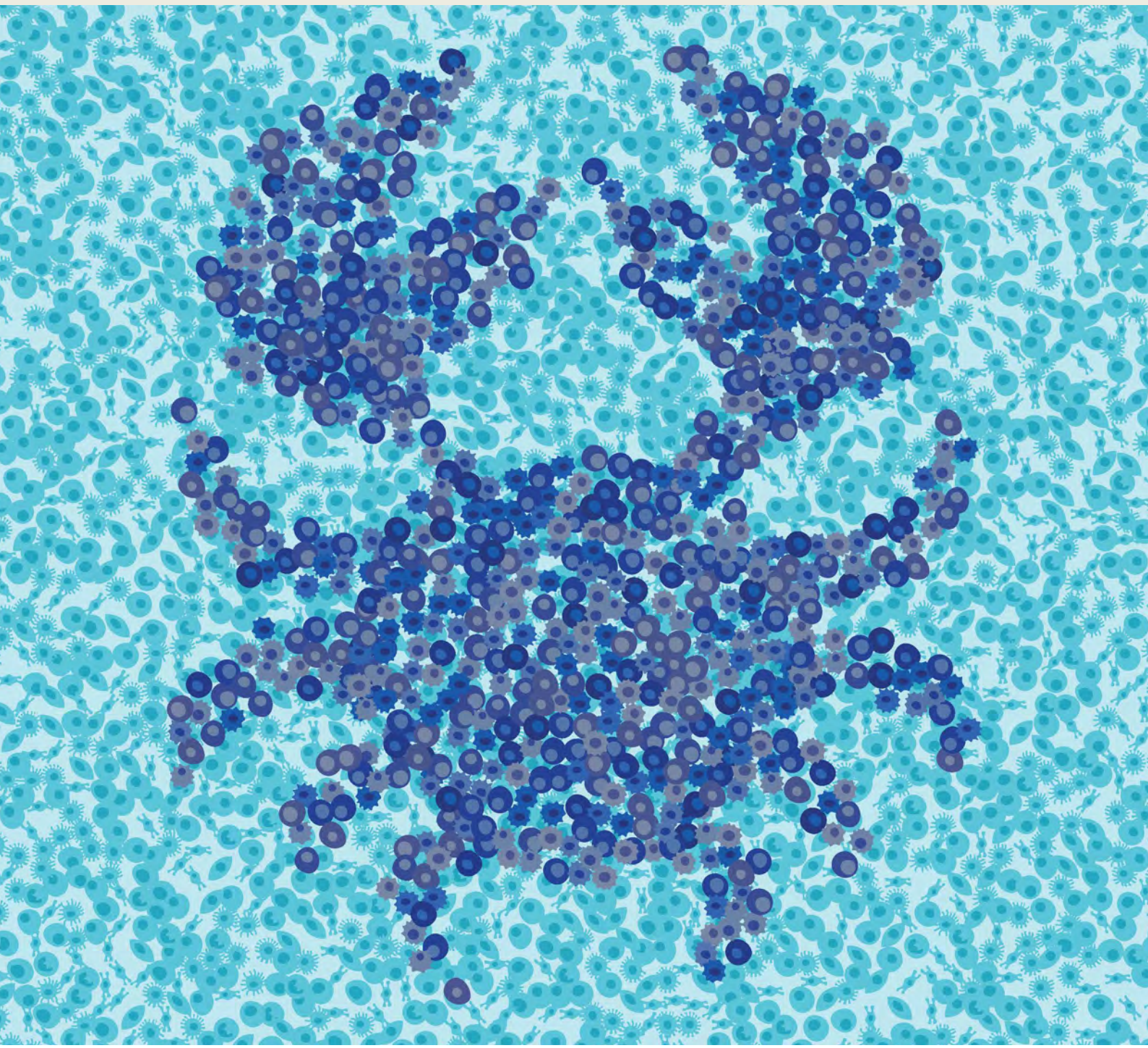
Cancer



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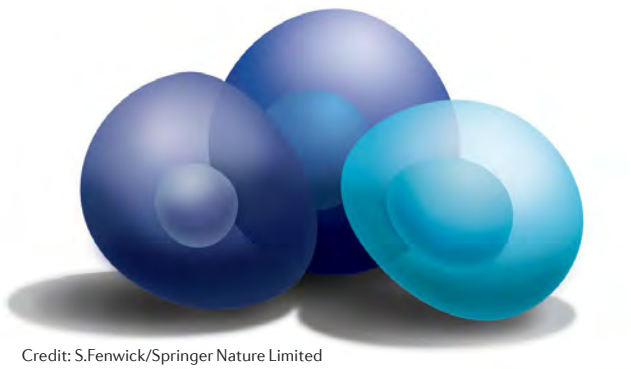
Cancer

MILESTONES



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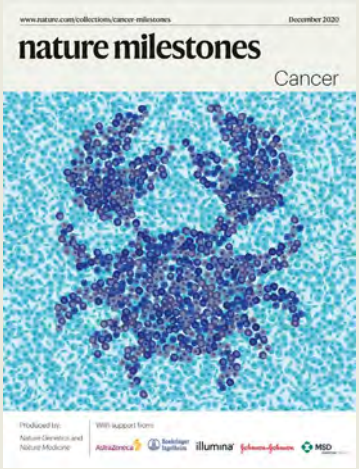

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SPRINGER NATURE

With little regard for borders, age, wealth or ethnicity, cancer has swept through human history and remains one of our biggest killers. In curating this Milestone collection, our aim was to pick up where our last Milestone project (https://www.nature.com/milestones/milecancer/timeline.html) left off and to showcase major advances in the understanding of cancer and the development of novel therapies that are improving patient survival. Although we have done our best to be comprehensive, we recognize that our list is by no means exhaustive.

In recent decades, understanding of the disease has developed at an astonishing pace. Our catalogues of the genetic (MILESTONES 7,11) and epigenetic (MILESTONE 10) aberrations underpinning tumour development are crystallizing. The adaptations used by tumour cells to breach cell-intrinsic (MILESTONES 5,6) and tissue-specific proliferative barriers, and establish malignant diaspora at secondary sites are better understood than ever before. Cancer cells can be profiled at unprecedented scale and resolution, increasingly in the context of their tissue and microbial (MILESTONE 13) microenvironments.

These discoveries have propelled the development of new treatments, most notably immunotherapies (MILESTONES 8,9), which are now a crucial part of the treatment armoury, alongside surgery, chemotherapy, radiotherapy and an expanding repertoire of targeted treatments (MILESTONES 4,12).

We hope that these Milestones will inspire optimism about the future of cancer research. We look forward to new approaches to tackle cancer types for which progress to date has been modest. We anticipate further improvements in the understanding of treatment resistance (MILESTONE 1) and metastasis, the process responsible for most cancer deaths. We also hope that technological innovations will drive powerful new strategies to detect and monitor cancer (MILESTONES 2,14).

As treatments become more sophisticated, so too must the strategies to ensure that the benefits of research are available to everyone. The socioeconomic disparities that disproportionately limit access to care must be overcome. From prevention (MILESTONE 3) to diagnosis and treatment—we must ensure that no patient is left behind.

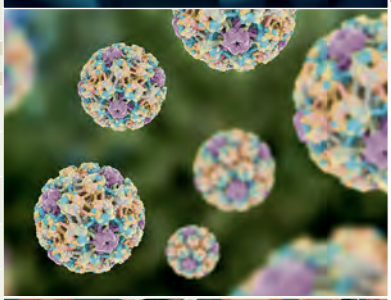
This project was made possible by the support of our colleagues in the Nature Editorial Cancer Community. We thank Javier Carmona and Ian Green for preparing the original proposal. In addition to the many editors who wrote these milestones, we extend our gratitude to Javier Carmona, Anna Dart, Iain Dickson, Linda Gummlich, Ulrike Harjes, Barbara Marte and Sarah Seton-Rogers for managing and editing individual milestones. We appreciate the support we have received from Rebecca Jones, Simon Fenwick, Chris Ryan and Maya Shani. Finally, we would like to thank our expert advisors and to acknowledge support from our sponsors and grant funders (AstraZeneca, Boehringer Ingelheim, Illumina, Johnson & Johnson and MSD). As always, Springer Nature takes complete responsibility for the editorial content.

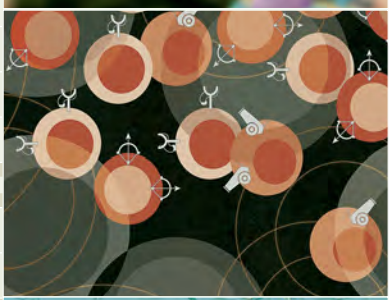
Safia Danovi, Senior Editor, *Nature Genetics*
Saheli Sadanand, Senior Editor, *Nature Medicine*

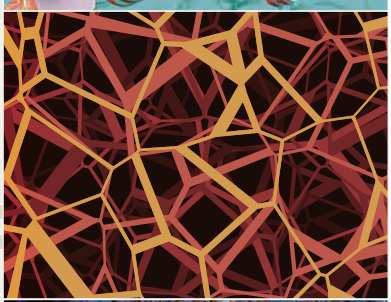
MILESTONES IN CANCER

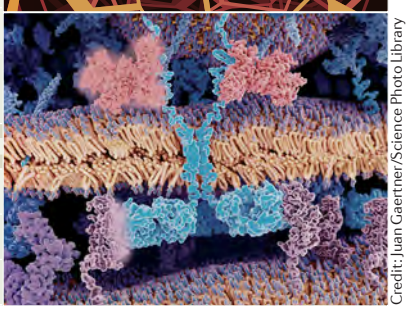
2000	Mitochondrial complex II mutations found in tumours
2001	Mechanisms of resistance to targeted treatment (MILESTONE 1)
	Nobel Prize awarded for "discoveries of key regulators of the cell cycle"
2003	Epidemiological link between cancer and obesity
2004	First epigenetic drug to gain FDA approval
	First antiangiogenic agent to gain FDA approval for cancer treatment
	Liquid biopsies for non-invasive diagnosis and monitoring of patients (MILESTONE 2)
	HPV vaccines to prevent cervical cancer (MILESTONE 3)
2005	Leveraging synthetic lethality for treatment (MILESTONE 4)
	Oncogene-induced senescence in premalignant tissues and cancer (MILESTONE 5)
2006	Metabolic adaptations in cancer (MILESTONE 6)
2008	First interim analysis published by The Cancer Genome Atlas
	First cancer whole-genome sequence (MILESTONE 7)
2009	Description of colorectal cancer organoids
	IDH1 mutations leading to the generation of 2-hydroxyglutarate
2010	Immune-checkpoint inhibitors from bench to bedside (MILESTONE 8)
	Engineering T cells to kill cancer cells (MILESTONE 9)
2011	Use of screening to decrease mortality from lung cancer
	Clearance of senescent cells by the immune system
2012	Epigenetic drivers of tumour initiation and progression (MILESTONE 10)
	Clonal diversity of tumour cells as a basis for cancer progression and treatment resistance (MILESTONE 11)
	Full-length single-cell mRNA sequencing of individual tumour cells
	Anti-tumour role of metabolite depletion
2013	Targeting 'undruggable' non-kinase proteins (MILESTONE 12)
	Gut microbiome influences on anti-tumour immune responses (MILESTONE 13)
2015	The Big Bang theory of cancer evolution is proposed
	Driver mutations found in healthy tissue
	First FDA approval for a combination of immunotherapies
2016	First FDA approval for an anti-PD-L1 inhibitor
2017	Potential of artificial intelligence in cancer diagnosis and monitoring (MILESTONE 14)
	First FDA approval of a treatment on the basis of tumour genomics alone
	First inhibitor of mutant IDH2 approved for clinical use
2018	Nobel Prize awarded for "discovery of cancer therapy by inhibition of negative immune regulation"
2019	Clinical trial of CAR T cells to target BCMA in patients with multiple myeloma
	Nobel Prize awarded for "discoveries of how cells sense and adapt to oxygen availability"
2020	Pan-cancer analysis of whole genomes
	Clinical trial of CD19-targeting CAR–Natural Killer cells in patients with $CD19^{+}$ cancers

Credit: Sciepro/Science Photo Library









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MILESTONE 1

Routes to resistance

By the turn of the millennium, drugs that selectively target driver genes had been developed. For instance, tyrosine kinase inhibitors, such as imatinib, had been demonstrated to lead to sustained and durable remission in patients with advanced chronic myeloid leukaemia (CML) by targeting *BCR-ABL*, a fusion gene with constitutive tyrosine kinase activity. Furthermore, in contrast to more conventional genotoxic treatments, these drugs were believed to cause fewer adverse effects. With such precise targeted therapies, hopes were high that this new generation of drug might represent the 'magic bullet' long sought after by patients and clinicians.

Unfortunately, the reality was not so simple. Although patients (even those in advanced stages or with complex molecular alterations) initially responded to these targeted drugs, a clinical trial of imatinib reported by Druker et al. showed that in patients with acute lymphoblastic leukaemia or with CML in lymphoid blast crisis, tumours eventually returned after daily treatment for a few weeks or months. The question of how cancer can adapt to specialized strategies that directly target essential cancer machinery still remained.

Mercedes E. Gorre, Charles Sawyers and collaborators set out to answer this question. Imatinib was known to work by binding the BCR-ABL kinase domain, thus blocking its function. Because previous work by Chin et al. had shown that cancers often require the activity of their primary oncogene, Gorre et al. wondered whether imatinib-resistant tumours might still be dependent on the *BCR-ABL* fusion gene. They reasoned that if the relapsed tumours were still dependent on *BCR-ABL*, then BCR-ABL signalling activity would be evident even after treatment. Because of the fast degradation of the BCR-ABL protein, they measured the phosphorylation of one of its downstream targets, CRKL. Indeed, in tumours from 11 patients with relapse, CRKL phosphorylation was nearly as high as that in untreated patients.

The next step was to identify what allowed BCR-ABL to remain active. The authors concluded that a cell-intrinsic factor was responsible, because relapsed cells isolated from patients still showed this oncogenic activity, thus suggesting that no extrinsic factors were involved. The authors examined changes in the *BCR-ABL* gene itself and found two strikingly distinct escape mechanisms in different patients.

The first resistance-related alteration could be understood as a brute-force, all-out approach to fight against the inhibitor: the tumours of three patients who relapsed had produced multiple copies of the *BCR-ABL* gene through gene amplification. The number of copies increased with subsequent rounds of treatment; however, in one patient, these amplifications disappeared after switching to another therapy, thus suggesting that the drug was selecting for clones bearing the amplification. In contrast, the second resistance mechanism required a single modification: a point mutation changing threonine 315 of ABL1 to isoleucine was found in six patients. Because this particular amino acid is critical for imatinib binding, the mutation abrogated binding to the drug. In contrast to the wild-type *BCR-ABL*, this mutant retained activity in cell lines, even after exposure to the drug.

This study, together with similar studies published shortly afterwards—including the discovery by Kobayashi et al. of *EGFR* mutations conferring resistance to gefitinib in lung cancer—illustrates several important aspects of cancer. On the one hand, these data show that cancer is an evolutionary process: under strong selective pressure, cells with adaptations allowing them to overcome the adverse environment will dominate. Such cells may actually already be present in the initial tumour—even seemingly homogeneous cancers can harbour genetically heterogeneous populations that have an edge in the 'arms race' against therapy, as described by Dagogo-Jack and Shaw. In addition, resistance can be achieved by markedly distinct but functionally convergent approaches. On the other hand, these studies have also unmasked one critical feature of cancers: certain genes and mutations remain essential drivers of tumour growth and survival. Therefore, knowing and targeting these central drivers continues to be an important clinical strategy, which is being used to develop new generations of clinically effective tyrosine kinase inhibitors.

Targeted therapies will remain an important part of the arsenal to combat cancers, especially when tested in different combinations that can circumvent resistance to a single drug. The roles of off-target, non-genetic mechanisms in this resistance are also starting to be acknowledged and will need to be addressed with correspondingly tailored approaches. As long as the design and use of these therapeutic strategies is guided by evolutionary principles, the hope is that drug resistance in patients will one day be predicted and sidestepped.

Ilse Valtierra, *Nature Communications*

ORIGINAL ARTICLE Gorre, M. E. et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 293, 876–880 (2001).
FURTHER READING Please visit the online article for a full list of further reading.

MILESTONE 2

Tracking cancer in liquid biopsies

Oncologists have long been aware that cancer cells disseminate through the bloodstream. In the early 2000s, substantial efforts were devoted to developing techniques for the reliable and sensitive detection of cancer cells and their components in bodily fluids. As cell-detection systems were optimized, several studies aimed to determine their clinical utility. A study published by Cristofanilli et al. in 2004 was the first to use the CellSearch platform to show that the number of circulating epithelial cells in the blood is markedly higher in women with metastatic breast cancer before starting systemic therapy than in women without breast cancer or with benign breast disease. In analysing survival outcomes, the investigators established the prognostic value of such differences: the durations of progression-free survival (PFS) and overall survival were significantly shorter in patients with cell counts above an established threshold at baseline and, more importantly, at the first follow-up visit during treatment. This study was the first to demonstrate the clinical relevance of circulating tumour cell enumeration for stratifying cancer patients.

Subsequent studies explored more specific approaches to identify the presence of tumour-derived material in blood, such as detecting tumour-related mutations in circulating tumour cells (CTCs). In patients with *EGFR*-mutated non-small-cell lung cancer, Maheswaran et al. have demonstrated the feasibility of using DNA extracted from CTCs for non-invasive monitoring of patients under therapy. Among patients in this study who received EGFR tyrosine-kinase inhibitors, the PFS duration was shorter in those carrying the resistance-related $EGFR^{T790M}$ alteration.

To overcome the technical challenges associated with purifying DNA from CTCs, several groups have focused on refining the detection of somatic mutations in DNA extracted from the cell-free fraction of human blood. Through sensitive detection of tumour mutations in a cohort of patients with colorectal cancer, pioneering work by Diehl et al. has shown an abrupt decrease in circulating tumour DNA (ctDNA) levels in blood samples from patients who had undergone complete surgical resection or chemotherapy. Moreover, the disease recurrence rates were significantly lower in patients with undetectable rather than detectable levels of ctDNA, thus providing the first evidence of the potential value of using ctDNA analysis as a tumour biomarker.

Credit: sorbetto

The diverse clinical applications that derived from analysis of circulating tumour material eventually led to the coining of the term 'liquid biopsy' by Pantel and Alix-Panabières in 2010. In subsequent investigations, researchers assessed the potential of liquid-biopsy tools for non-invasive monitoring of response to therapy in patients with cancer. In a landmark study by Dawson et al. in 2013, serial blood samples from women with metastatic breast cancer undergoing treatment were collected, and CTC quantification and ctDNA analysis were compared side by side for disease monitoring. This work showed that ctDNA has superior sensitivity to that of CTCs as a cancer biomarker and, crucially, changes in ctDNA levels closely paralleled treatment responses: increased ctDNA levels were seen in 89% of the women with progressive disease.

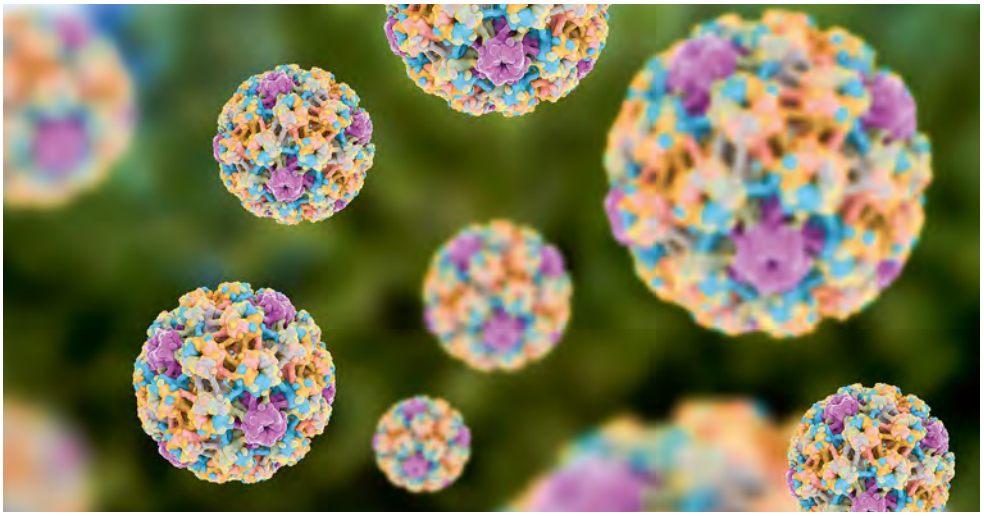
> One of the next challenges in this field will be incorporating liquid biopsies into routine cancer screening protocols to facilitate early cancer diagnosis

The results of another necessary comparison were published a year later by Bettegowda et al. Analysis of patients with cancers across 14 different tissues of origin revealed that ctDNA can be detected in the blood of most patients with solid tumours outside the brain. For some malignancies studied, the percentage of patients with detectable ctDNA was low, thus underscoring the need for developing comprehensive gene panels for liquid-biopsy assays. Expectedly, most patients with metastatic disease had detectable ctDNA; however, ctDNA was also found in a substantial proportion of patients with localized cancers. This observation confirmed that cancer cells and cancer-derived DNA can enter the bloodstream at any stage of disease progression, as had already been proposed, thus drawing new attention to a role for liquid biopsies in enabling early cancer detection.

The encouraging initial results from these and other studies are now prompting clinicians to increasingly use liquid biopsies for a range of clinical applications, including predicting the risk of disease recurrence, as reported by Tie et al., matching patients to treatments while minimizing surgical procedures to obtain tissue biopsies, and tracking the presence of resistance-related mutations. One of the next challenges in this field will be incorporating liquid biopsies into routine cancer screening protocols to facilitate early cancer diagnosis. The feasibility of this approach has already been demonstrated in a pilot intervention by Chan et al. focused on the detection of Epstein–Barr virus–related nasopharyngeal carcinoma, and in a pan-cancer study by Lennon et al. combining positron emission tomography–computed tomography with the detection of biomarkers in blood (including ctDNA).

Diana Romero,
Nature Reviews Clinical Oncology

ORIGINAL ARTICLE Cristofanilli, M. et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N. Engl. J. Med.* **351**, 781–791 (2004).
FURTHER READING Please visit the online article for a full list of further reading.



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MILESTONE 3

When cancer prevention went viral

Cancer prevention strategies are theoretically appealing although often difficult to implement, owing to the multifactorial pathogenesis of most cancers. The possibility of a notable exception first emerged in 1983, when Harald zur Hausen and others confirmed the presence of a specific human papillomavirus (HPV) subtype (HPV-16) in biopsy samples from several cohorts of patients with genital cancers.

Further research followed, and in 1999, HPV was confirmed as being not only present and involved in the aetiology, but also a necessary cause of virtually all cervical cancers. By that time, attempts to develop an HPV vaccine were already underway, and several technical hurdles had been overcome, including the availability of 'virus-like particles' (VLPs), which safely and efficiently induced infection-preventing antibody responses in animal studies.

Results from the first clinical trial of an HPV vaccine, involving a VLP-derived vaccine targeting HPV-16, were published in 2002. The efficacy results were deemed unequivocal by most experts: all 41 cases of persistent HPV-16 infections in a cohort of 2,392 18–23-year-old women were observed in the placebo group.

Another trial, by Harper et al., published in November 2004, provided the first evidence that HPV vaccination might reduce cervical cancer risk: Cervarix, a bivalent vaccine for HPVs 16 and 18, was found to reduce the risk of associated cervical abnormalities from 4.9% to 0.4%. A total of seven women had cervical intraepithelial neoplasia, including one in the vaccine group, who was later found to have a persistent HPV-51 infection. This experience highlighted the potential advantage of a vaccine covering a broader range of HPV subtypes.

Subsequent trials further confirmed the efficacy of HPV vaccination, including that with Gardasil, a quadrivalent vaccine targeting HPVs 6, 11, 16 and 18. Gardasil later became the first HPV vaccine to receive regulatory approval, by the US Food and Drug administration (FDA) in June 2006, for use in girls and women 9–26 years of age. A second approval followed, this time of Cervarix from the European Medicines Agency (EMA), which included vaccination for the prevention of anal cancer in males in the same age group. The FDA and EMA subsequently authorized Gardasil-9, which provides immunity against nine HPV strains, in 2014 and 2015, respectively.

> “Despite these apparent successes, the potential of HPV vaccination is only beginning to be realized.”

HPV vaccination of males serves two purposes: vaccinated men are presumably less likely to transmit HPV to any sexual partners, and furthermore, vaccination is likely to confer some protection against other HPV-positive cancers, including those of the penis, oropharynx, oral cavity/larynx and anus. The latter possibility is supported by prospective data: Gardasil approximately halved the risk of grade 2 or 3 vaccine type HPV-related anal intraepithelial neoplasia in a cohort of men who have sex with men, a population known to have a particularly high risk of anal infections and cancers.

The long latency period between initial HPV infection and cervical cancer makes any reduction in cancer risk difficult to assess directly. Nonetheless, a registry study from Sweden, where HPV vaccines have been available since 2006, has revealed that the incidence of invasive cervical cancer among vaccinated women was approximately half that of nonvaccinated women. The incidence was 88% lower for those vaccinated below 17 years of age. Despite these apparent successes, the potential of HPV vaccination is only beginning to be realized. Many economically developed countries continue to lack universal HPV vaccination programmes, and vaccine availability is often limited to health centres, rather than schools and other community settings. Increasing public mistrust in vaccines might pose further challenges to implementation. Other challenges include the development of HPV vaccination programs for less economically developed countries, where cervical cancer is often the most common cause of cancer mortality. The emergence of generic HPV vaccines and the incidental finding that even single-dose vaccination provides some protection against HPV infection will hopefully enable wider implementation and might assist in addressing these challenges.

Peter Sidaway,
Nature Reviews Clinical Oncology

ORIGINAL ARTICLE Harper, D. M. et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet* **364**, 1757–1765 (2004).
FURTHER READING Please visit the online article for a full list of further reading.

Credit: Richard Drury

MILESTONE 4

A licence to kill

Synthetic lethality, a term coined by Theodore Dobzhansky in 1946, arises when co-occurring mutations in two genes kill cells, whereas mutation of either gene alone does not and may elicit a milder phenotype. This phenomenon was first described by Calvin Bridges in 1922 in genetic experiments in *Drosophila melanogaster*. Mechanisms underlying synthetic lethality are now known to include genetic and non-genetic redundancies, buffers and adaptation.

A few decades later, the concept of synthetic lethality was applied to cancer research, ultimately leading to the approval of new therapies. Rapidly dividing cells had long been known to be susceptible to drug-induced DNA damage, suggesting that DNA-repair inhibitors might selectively kill cancer cells. By the 1980s, inhibitors of the DNA-repair poly(ADP)–ribose polymerase (PARP) enzymes had been shown to kill cancer cells more efficiently in concert with DNA-damaging agents than as single agents. Then, in a seminal 1997 article, Leland Hartwell, Stephen Friend and colleagues suggested that synthetic-lethality relationships could lead to new anticancer drug targets, and genetics might therefore offer a rational approach to drug discovery. Because genetic and drug screens were then largely limited to model organisms, the researchers used a *Saccharomyces cerevisiae* screen, focusing on defects in DNA-repair and cell-cycle genes. Indeed, some chemotherapeutic drugs selectively killed cells with specific genetic mutations.

> “The results strikingly revealed a large therapeutic window (or index) both in vitro and in mice.”

Building on these findings, two landmark studies in 2005 by Alan Ashworth's group in collaboration with KuDOS Pharmaceuticals Ltd. and the groups of Thomas Helleday and Nicola Curtin demonstrated that human cancer cells with mutations in the DNA-repair tumour-suppressor genes *BRCA1* and *BRCA2* are selectively sensitive to PARP inhibitors. Loss of the base-excision-repair enzyme PARP1 increases DNA lesions, such as collapsed replication forks, which can normally be repaired through homologous recombination (HR). Therefore, the teams reasoned that defects in BRCA1 or BRCA2, which participate in HR, might be synthetically lethal with the loss of PARP1 or PARP inhibition. Indeed, cells with deletion of *B*RCA1 or BRCA2 (or other HR genes) were viable but died after PARP-inhibitor treatment. The results strikingly revealed a large therapeutic window (or index) both in vitro and in mice. The findings were notable because people with *BRCA1* or *BRCA2* germline mutations are predisposed to breast, ovarian and prostate cancer, and the tumours that develop exhibit loss of BRCA function and impaired HR. Subsequent observations from the groups of Alan Ashworth and Toshiyasu Taniguchi showed that resistance to PARP inhibitors or platinum (which also targets HR) can arise due to unexpected secondary function-restoring alterations in *BRCA2* providing further evidence of a synthetic-lethal relationship.

As a direct result of this work, in 2014, the PARP inhibitor olaparib became the first targeted therapy for the treatment of patients with ovarian cancer with germline *BRCA1/2* mutations to be approved by the European Medicines Agency (EMA) and US Food and Drug Administration (FDA). Olaparib and three other PARP inhibitors have since been approved for several other malignancies, some with loss of BRCA1/2 function (breast, pancreatic and prostate cancer).

Beyond associations with specific genetic mutations, drugs may synergize, such that treatment with one cancer drug exposes a vulnerability to a second drug. In 2012, René Bernards and colleagues studied why melanoma cells with the activating V600E alteration in the kinase BRAF are sensitive to BRAF inhibitors, but colorectal cancer cells with the same mutation are not. In a synthetic-lethality screen, BRAF inhibition in colorectal cancer cells exposed a sensitivity to the concomitant loss or inhibition of the receptor tyrosine kinase EGFR, both in vitro and in vivo. This synergy arose from rapid feedback activation of EGFR signalling after BRAF-inhibitor treatment. In contrast, melanoma cells express little EGFR; therefore, BRAF inhibitors did not stimulate EGFR activation. These findings led to the 2020 EMA and FDA approval of combination treatment with the BRAF inhibitor encorafenib and the EGFR-targeting antibody cetuximab for *BRAF*-mutant metastatic colorectal cancers.

Examples of cancer-specific synthetic-lethal relationships in mammalian cells and strategies to systematically discover and exploit synthetic-lethal interactions for cancer therapy were discussed in an influential 2005 review by William Kaelin, and many still hold true. The application of synthetic lethality has rapidly advanced, and sophisticated, high-throughput genetic and drug screens, and more recently CRISPR–Cas9 technology are often used. Drugs have been approved for more indications, and many more clinical trials based on the principles of synthetic lethality are underway. Current areas of exploration include cell-intrinsic mechanisms, such as the BRCA–PARP and BRAF–EGFR interactions, and vulnerabilities mediated by the tumour microenvironment, such as combinations of targeted drugs with immunotherapies.

Barbara Marte, *Nature*

ORIGINAL ARTICLES Bryant, H. E. et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature*. **434**, 913–917 (2005) | Farmer, H. et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*. **434**, 917–921 (2005) | Prahallad, A. et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature*. **483**, 100–103 (2012).
FURTHER READING Please visit the online article for a full list of further reading.

MILESTONE 5

Sitting on the fence

Picture this: an unattended stew is boiling over and splatters everywhere. What could we do? We could put on a lid, remove the pot from the heat, and leave it to cool. Similarly, primary mammalian cells appear to have found ways to handle their own undesirable spread. Cellular senescence can serve as a break in excessive proliferation, providing an initial barrier, and eventually protection, against tumorigenesis.

Cellular senescence is provoked by either an intrinsic mitotic counter (replicative senescence) or extrinsic factors, such as oxidative stress or DNA damage at any point in the cell's replicative history (premature senescence). The latter can also be driven by activated oncogenes, such as those in the RAS family, and is better known as oncogene-induced senescence (OIS). In the late 1990s, findings from cultured cells linked OIS to two major cell-signalling pathways that are often disrupted in cancer: INK4A–RB and ARF–p53. However, at that point, whether OIS was an authentic anticancer process or an artefact of imposed oncogene expression in cells experiencing 'culture shock' was unclear.

In 2005, four groups reported remarkable in vivo evidence of the existence of OIS in several mouse and human premalignant tissues. They presented a novel set of senescence markers and provided a sneak peek into the molecular groundwork. Although unified in their finding that OIS is tumour suppressive, these studies demonstrated that pathways underlying OIS are dependent on the tumour tissue and oncogenic insult.

Until 2005, senescence-associated β-galactosidase was the 'gold standard' in vivo marker for senescence. Collado et al. identified a small set of genes with expression profiles correlating with the KRAS-V12-induced senescence phenotype and used these to show that senescent cells exist among premalignant adenomas but not malignant adenocarcinomas in lung, skin and pancreatic tissues. Confirming the discoveries in preneoplasia, Michaloglou et al. characterized the telomere- and p53-independent BRAF-V600E-induced senescence observed in skin moles (nevi) but not melanoma cells. In contrast, Chen et al. showed that p53 is crucial for OIS in premalignant prostate tissue in vitro and in vivo when the tumour suppressor PTEN is lost. Loss of p53 and PTEN inhibits any protection from prostate cancer development, but this type of induced senescence is reversed by deletion of p53 regulators.

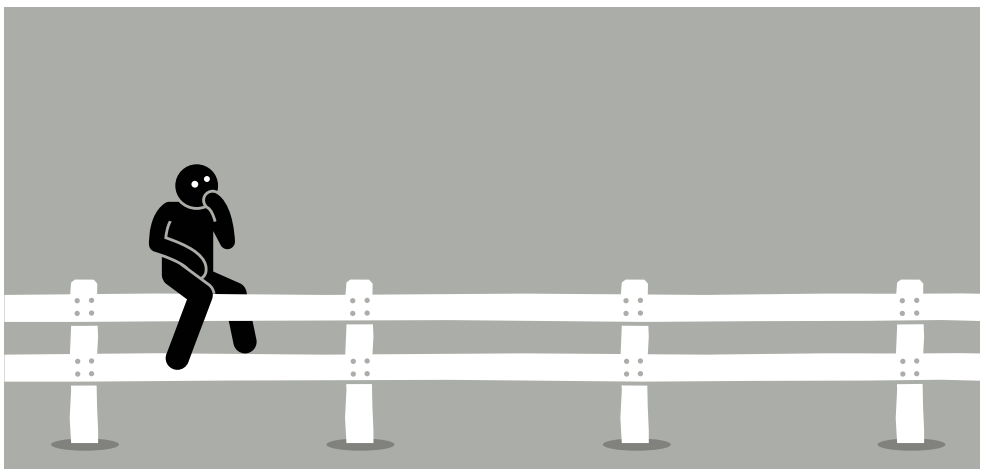
Senescent cells often show unusual chromatin foci of tightly packed DNA. Braig et al. researched the epigenetic contributions and found that NRAS-induced lymphocyte senescence is dependent on the histone methyltransferase SUV39H1 and the tumour suppressor RB, which jointly promote DNA compaction mediated by methylation of histone H3 Lys 9 (H3K9me); this process is crucial for triggering H3K9me-mediated senescent growth arrest and thus providing an initial barrier to lymphoma development.

> "Research in the past decade has exposed the multifaceted and highly dynamic nature of senescence and its star players"

Do senescence triggers interact? Two subsequent studies in 2006 provided robust evidence that OIS (induced via the oncoproteins HRAS-V12, MOS, CDC6 or cyclin E) is associated with signs of DNA-replication stress, thereby establishing that OIS is a direct consequence of a vigorous DNA-damage-checkpoint response, after DNA hyper-replication and the formation of double-strand breaks. In addition, inflammatory mediators appear to be a crucial aspect in the barrier network. Cells undergoing OIS exhibit a senescence-associated secretory phenotype (SASP). In 2008, the secretion of multiple chemokines and interleukins (including IL-6 and IL-8) was found to maintain growth arrest, thereby stabilizing the system, whereas SASP factors may act as growth promoters in other settings.

Is the induction of senescence the long-awaited tool for cancer therapy? Sadly, life is rarely that simple, and research in the past decade has exposed the multifaceted and highly dynamic nature of senescence and its star players. Although therapy-induced senescence can improve long-term outcomes, it has also been found to cause relapse, enhanced self-renewal and adverse reactions to cancer treatment. Consequently, the elimination of senescent cells has recently emerged as a sensible therapeutic strategy. Current investigations are underway to explore the clinical potential and benefits for cancer patients.

Linda Gummlich, *BMC Cancer*



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ORIGINAL ARTICLES Collado, M. et al. Senescence in premalignant tumours. *Nature* **436**, 642 (2005). | Braig, M. et al. Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature* **436**, 660–665 (2005). | Michaloglou, C. et al. BRAF^{E600}-associated senescence-like cell cycle arrest of human naevi. *Nature* **436**, 720–724 (2005). | Chen, Z. et al. Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis. *Nature* **436**, 725–730 (2005).

FURTHER READING Please visit the online article for a full list of further reading.

MILESTONE 6

Not a simple switch

Credit: peepo

Cells respire—they consume oxygen and glucose to produce energy in the form of ATP. When oxygen is not available, cells in differentiated tissues break glucose down into lactate (through glycolysis) for energy production. The 'Warburg effect' describes Otto Warburg's observations in the 1920s that malignant tumour cells mainly perform glycolysis even in the presence of oxygen (aerobic glycolysis), thus resulting in high lactate secretion. These observations led him to believe that during malignant transformation, initial irreversible damage in respiration was followed by upregulation of glycolysis to replace the loss of respiration energy. According to Warburg, the origin of cancer was purely based on metabolic alterations. We know today that Warburg was not quite right: oncogenic signalling fundamentally contributes to malignant transformation, partly by regulating metabolism.

By the 1990s, the presence of aerobic glycolysis in some tumours had been clinically exploited for cancer detection through fluorodeoxyglucose–PET imaging. Yet the molecular basis of the Warburg effect remained ill-defined. In 1997, Chi V. Dang and colleagues reported that the glycolytic enzyme lactate dehydrogenase A (LDHA), which catalyses the final step of glycolysis, is a transcriptional target of the oncoprotein MYC. They showed that overexpression of MYC or LDHA resulted in greater aerobic lactate production in cultured fibroblasts. LDHA was also necessary for MYC-induced anchorage-independent cell growth. These findings provided a molecular basis for the Warburg effect, which, contrary to Warburg's hypothesis, involved an oncogene frequently altered in cancer.

The link of cancer-driving gene mutations to the Warburg effect was corroborated on the level of tumour suppressors. Cellular respiration relies on the tricarboxylic acid (TCA) cycle as well as the electron-transport chain (ETC) coupled to oxidative phosphorylation (OXPHOS) in the mitochondria. Indeed, the tumour-suppressor protein p53 was found to participate in controlling the balance between glycolysis and OXPHOS, as reported by Paul M. Hwang's and Karen Vousden's groups in 2006 (See *Mitochondrial complex II mutations found in tumours* on the interactive timeline).

The TCA cycle also generates molecules needed for biomass production. Considering that glycolysis is a relatively inefficient way to produce energy, Craig B. Thompson's group, in one of the first cancer metabolism studies to use carbon-13 isotope tracing, explored the metabolic underpinnings of the Warburg effect. They found that cancer cells use glucose-derived TCA-cycle intermediates in synthetic pathways (particularly fatty acid synthesis). Cells also require continuous replenishment of the TCA cycle with a non-glucose carbon source—a process achieved through uptake and metabolism of the amino acid glutamine. These and other data led Matt G. Vander Heiden, Lewis C. Cantley and Thompson to propose a model in which metabolism in cancer cells is adapted to optimize access to nutrients and their incorporation into biomass, thereby generating a growth advantage.

Further shaking Warburg's hypothesis of irreversibly damaged mitochondria in cancer, Navdeep S. Chandel's group showed that mitochondrial metabolism and ETC function are required for cancer cell growth induced by the oncoprotein KRAS in vitro and in vivo. Moreover, glucose metabolism in the pentose-phosphate pathway (PPP) was found to be crucial for oncogenic KRAS-induced growth, through generating nucleotide precursors as well as NADPH. PPP-derived antioxidants are also linked to promoting cancer cell survival, as shown by Joan Brugge's group, thus highlighting the importance of antioxidant defence in cancer growth.

Research in the 2010s led the field further away from Warburg's path, showing substantial heterogeneity in cancer metabolism, and highlighting the oncogenotype and tissue environment as key determinants. Eileen White's and Alec Kimmelman's groups revealed that oncogenic KRAS-driven cancer cells depend on autophagy, a catabolic pathway that degrades intracellular organelles and macromolecules. Autophagy maintains mitochondrial metabolism and redox control, and allows cancer cells to maintain growth in nutrient-scarce environments. David M. Sabatini's and Cantley's groups have reported amplification of the gene encoding the rate-limiting enzyme in the serine synthesis pathway in certain cancer types, thus making the growth of these cancers dependent on serine and glycine metabolism. Crucially, J. Michael Bishop's group has shown in mice that metabolism in MYC-driven liver cancer not only differs from metabolism in MYC-driven lung cancer, but also differs from metabolism in liver cancer driven by the oncoprotein MET. In humans, Ralph J. DeBerardinis and colleagues have observed metabolic heterogeneity among tumours across patients and within the same patient as well as among regions within the same tumour.

This complexity signifies that the cancer metabolism field has long outgrown the confines of the hypothesis that Warburg proposed nearly a century ago. Since then, seminal findings have been reported by many groups. Although research in the past two decades has largely focused on primary tumours, insights into metabolism during metastasis, in the tumour microenvironment, and in the systemic context, are rapidly advancing. To date, only a limited number of agents targeting cancer metabolism have been successfully applied in the clinic (See *IDH1 mutations lead to the generation of 2-hydroxyglutarate* and *FDA approval of enasidenib for acute myeloid leukaemia* on the interactive timeline), but this is likely to change in the future.

Ulrike Harjes, *Nature Reviews Cancer*

ORIGINAL ARTICLES Shim, H. et al. c-Myc transactivation of *LDH-A*: implications for tumor metabolism and growth. *Proc. Natl Acad. Sci. USA* **94**, 6658–6636 (1997) | Matoba, S. et al. p53 regulates mitochondrial respiration. *Science* **312**, 1650–1653 (2006) | Bensaad, K. et al. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. *Cell* **126**, 107–120 (2006).
FURTHER READING Please visit the online article for a full list of further reading.

MILESTONE 7

Sequencing the secrets of the cancer genome

The advent of next-generation sequencing (NGS) in the first decade of the twenty-first century heralded a major change in cancer research. Similarly to how electronic miniaturization transformed computers from room-sized appliances into smartphones that fit in the palm of a hand, the progress from low-throughput Sanger sequencing to high-throughput NGS allowed an entire genome to be sequenced by an individual research group, in contrast to the globe-spanning industrial effort required by the Human Genome Project only a few years earlier. By that time, cancer's status as a genetic disease was well established, and catalogues of oncogenes and tumour suppressors were available. However, the number of mutations in any given tumour, as well as the full extent of the 'cancer-gene census', remained unknown; these and other questions could finally be answered with the newly developed sequencing technologies.

The first cancer whole-genome sequence, of an acute myeloid leukaemia (AML) from a woman in her mid-50s, was published in 2008 by Ley et al. This karyotypically normal, highly pure case was carefully selected to ensure adequate input material for sequencing and to facilitate interpretation of the results. Two samples from this patient were sequenced with Solexa technology on an Illumina Genome Analyzer: primary tumour and normal skin tissue.

Single-nucleotide variant (SNV) analysis demonstrated the importance of including the matched normal sample: 3.8 million SNVs were identified in the tumour sample, of which 2.6 million also occurred in the skin and were thus categorized as germline variants. After further filtering, a total of eight SNVs were validated as novel somatic variants with a predicted effect on gene function. These eight genes had roles in functionally relevant pathways—cellular signalling (*PTPRT*, *KNDC1*, *ADGRA1*, *GPR183* and *GCOM2*), cell adhesion (*CDH24* and *CDHR2*) and transmembrane transport (*SLC15A1*)—thus demonstrating that genome sequencing can identify novel candidate cancer genes within key, potentially therapeutically targetable tumourigenic pathways. Most intriguingly, none of these eight somatic SNVs were observed in any of a cohort of 187 AML tumours, thus suggesting substantial heterogeneity in genes affected by mutation in a tumour type.

Beyond identifying a set of novel candidate cancer genes, this study attempted to track cancer evolution by comparing variant allele frequencies in amplicon sequencing data for ten somatic mutations (the eight SNVs, an *FLT3* internal tandem duplication and an *NPM1* insertion) in primary and post-relapse samples. This analysis suggested that nine of ten mutations were heterozygous in all tumour cells, but the last mutation—the *FLT3* internal tandem duplication—appeared to be subclonal and was suggested to be the most recent mutation.

In the following year, three additional cancer genomes from a metastatic breast tumour, and from lung and metastatic melanoma cell lines were published. These were distinctly different from the AML results in terms of the number of somatic mutations observed: 32 in breast cancer, and 33,345 and 22,910 in the melanoma and lung cell lines, respectively. Notably, the mutations identified in the breast tumour were largely mutually exclusive from those found in an extended series of 192 tumours, as observed for the AML genome. The mutational signatures of exposure to tobacco smoke (C>A) and ultraviolet light (C>T) were detected in the lung and melanoma genomes, respectively.

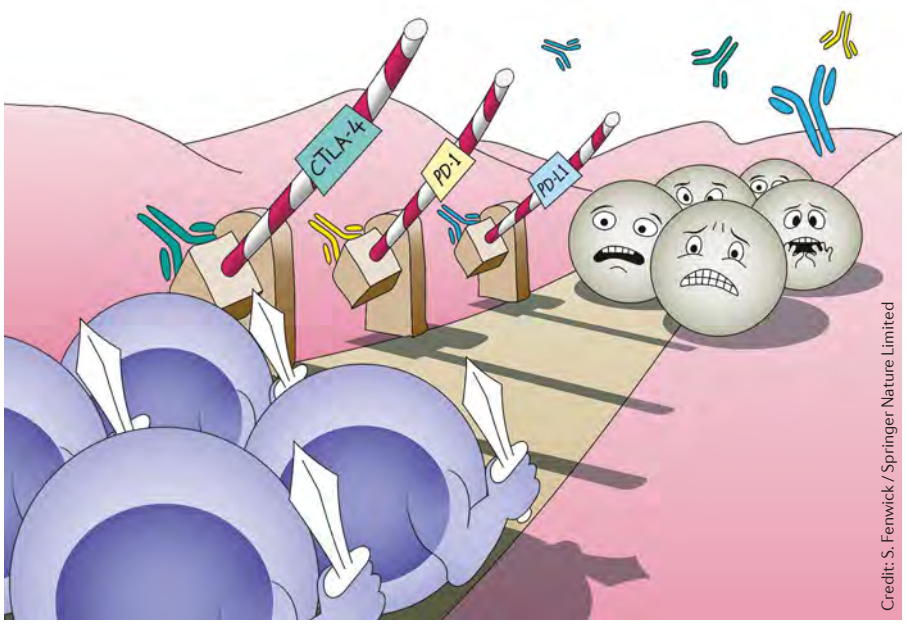
Credit: zmeel

Together, these four studies suggested that cancers have substantial genetic heterogeneity, both within and between individual tumour entities. Most importantly, these findings suggested a clear path for future research: whole-genome sequencing, and related techniques that profile transcriptomes and epigenomes, would need to be applied at scale to gain a better understanding of cancer's complex genetic basis and to provide insights into how these genetic discoveries might be clinically applied.

Large-scale studies such as The Cancer Genome Atlas (TCGA) and the Pan-Cancer Analysis of Whole Genomes (PCAWG) have now sequenced tens of thousands of cancer genomes across a wide variety of tumour types. The insights gained from these large cohorts include the identification of many more cancer genes associated with previously unanticipated cellular processes such as metabolism (*IDH1* and *IDH2*) and epigenetic regulation (*EZH2* and *PBRM1*), and the confirmation that most somatic mutations in tumours are not highly recurrent (the 'long tail' of mutation frequency). They have ignited the study of cancer evolution (MILESTONE 11), based on phylogenies of somatic mutations inferred through sequencing, which has shed light on the complex dynamics of tumourigenesis. Finally, the analysis of mutational signatures has identified many previously known and novel mutagenic processes, as well as the genomic signatures of prior exposures. Although the past decade has unequivocally demonstrated the value of genome sequencing in basic cancer research, clinical use remains restricted to well-resourced institutions equipped with the expertise to generate and interpret genomic data, thus suggesting that, at least for patients, the greatest impact of sequencing may be yet to come.

Michael Fletcher, *Nature Genetics*

ORIGINAL ARTICLE Ley, T. et al. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature* **456**, 66–72 (2008).
FURTHER READING Please visit the online article for a full list of further reading.



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MILESTONE 8

Unleashing the immune system against cancer

Efforts to harness the immune system for cancer therapy can be traced back over 100 years; however, cancers develop under constant immunosurveillance and therefore evolve diverse mechanisms of immunosuppression, which have proven difficult to overcome. A breakthrough that eventually led to the establishment of immunotherapy as a new pillar of cancer care was made in 1996, when James Allison and colleagues demonstrated that antagonistic antibodies targeting CTLA-4, a receptor known to suppress T cell activation, could induce tumour rejection in mice. This finding revealed the contribution of 'immune checkpoints' with physiological roles in self-tolerance to cancer immune evasion and simultaneously illuminated the therapeutic potential of immune-checkpoint inhibitors (ICIs). In 2003, Allison and collaborators provided clinical proof of this therapeutic concept in a first-in-human study of ipilimumab, an antibody to CTLA-4, in patients with melanoma or ovarian cancer.

Around the same time, preclinical work by Lieping Chen, Tasuku Honjo, Gordon Freeman, Arlene Sharpe and others characterized a different immune-checkpoint ligand, PD-L1, which can be found on cancer cells themselves (unlike CTLA-4 ligands, which are restricted to professional antigen-presenting cells). Furthermore, Chen and Honjo demonstrated that PD-L1 has a direct role in tumour immune evasion and can be targeted therapeutically with antibodies. Previously, Honjo had also identified PD-1, the receptor for PD-L1, on T cells. These advances spurred the development of a new class of ICIs targeting the PD-1–PD-L1 axis. In 2010, the first-in-human study of nivolumab, an antibody to PD-1, revealed durable regressions of several tumour types as well as a correlation between responsiveness and tumoural expression of PD-L1, which eventually proved to be a useful, albeit imprecise, predictive biomarker of response.

2010 witnessed a second landmark in the development of ICIs when ipilimumab became the first agent shown to prolong overall survival (OS) in patients with advanced-stage melanoma (to 10 months from a median of approximately 6 months). Accordingly, in March 2011, ipilimumab made history as the first ICI to be approved by the US FDA, thus validating the approach to immunotherapy originally proposed by Allison.

As a clearly immunogenic tumour type, melanoma became a key focus for the development of ICIs, resulting in rapid clinical advances. In 2013, objective response rates of up to 52% were achieved in a phase I trial of pembrolizumab, an antibody to PD-1. Responses were observed regardless of prior exposure to ipilimumab, hinting at distinct—and therefore potentially complementary—mechanisms of action. In fact, co-treatment with ipilimumab and nivolumab was already being tested and, in 2013, a phase I trial revealed that 53% of patients treated at the maximum doses had ≥80% tumour reductions. In 2014, pembrolizumab became the first approved PD-1 inhibitor, for ipilimumab-refractory melanoma, followed by approval of nivolumab in this setting less than 4 months later. Within a year, both pembrolizumab and the ipilimumab plus nivolumab combination had been approved in the frontline setting after superiority over ipilimumab was demonstrated in phase III trials. Remarkably, the median OS of patients treated with the combination was >5 years, emphasizing the unprecedented potential of ICIs to induce durable remission of metastatic cancers.

2015 also marked the start of a rapid expansion in approvals of PD-1 inhibitors to other tumour types, starting with non-small-cell lung cancer (NSCLC)—another malignancy in which ICIs have dramatically reduced mortality. In 2016, atezolizumab became the first anti-PD-L1 antibody to join the arsenal of ICIs, with demonstrated activity against urothelial carcinoma. ICIs have now entered the treatment armamentarium for at least 17 advanced-stage malignancies, in many cases bringing the possibility of long-term survival and perhaps even cure for a subset of patients.

ICIs are, however, not a panacea. Although generally better tolerated than other antineoplastic agents, ICIs come with a risk of diverse and potentially serious immune-related adverse events (irAEs). Moreover, in almost all cancers, responses are limited in frequency and duration. Initial studies revealed aberrations affecting interferon signalling (*JAK1/2* mutations) and/or the antigen-presentation machinery (*B2M* mutation) as mechanisms of resistance. By contrast, cancers with certain mutational signatures generate large numbers of non-self epitopes (neoantigens) and are therefore particularly sensitive to ICIs. This concept was illustrated by the approval of pembrolizumab for microsatellite instability-high/mismatch-repair-deficient cancers regardless of histology—the first tissue-agnostic approval—in 2017 and for cancers with a high mutational burden in 2020.

Undoubtedly, ICIs have revolutionized cancer therapy, as reflected in the awarding of the 2018 Nobel Prize in Physiology or Medicine to Allison and Honjo. To further exploit the therapeutic potential of ICIs, these agents are being explored for earlier-stage cancers, with approved adjuvant and consolidative indications following definitive local therapy for melanoma and NSCLC, respectively. These agents are also being combined with various other treatments, resulting in approvals involving chemotherapies and anti-angiogenics. However, important issues must be addressed to further improve patient outcomes, particularly by improving on the currently suboptimal predictive biomarkers and by mitigating irAEs.

David Killock, *Nature Reviews Clinical Oncology*

ORIGINAL ARTICLE Hodi, F. S. et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* **363**, 711–723 (2010).
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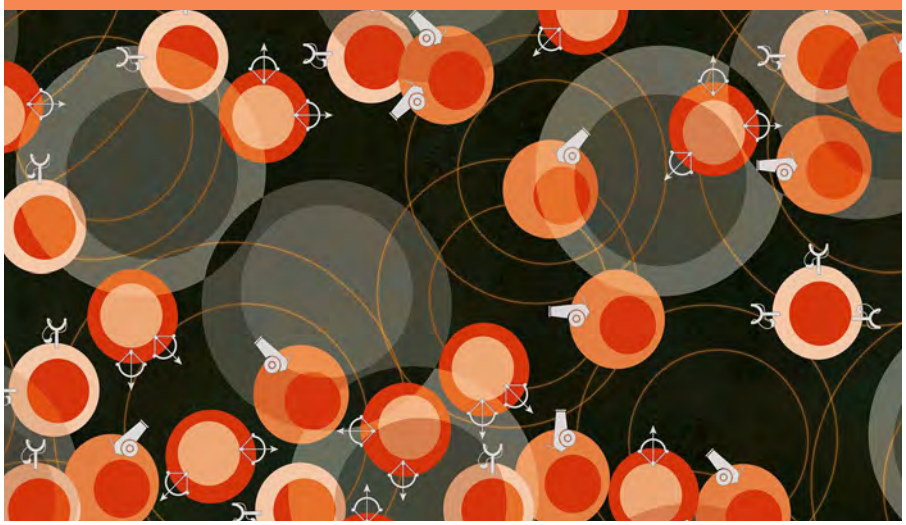
MILESTONE 9

Engineering armed T cells for the fight



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T cells, which can efficiently identify and kill infected cells, can also kill cancer cells if they recognize them. Adoptive cell therapies (ACT) leverage this cytotoxic capacity of T cells to eradicate tumours.

The first successful clinical applications of ACT for cancer treatment occurred in the 1980s. In these pioneering studies by Steven Rosenberg, tumour-infiltrating lymphocytes (TILs) were isolated from patients with melanoma, then activated and expanded before reinfusion into the patients. In the first published gene therapy clinical trial, TILs engineered to express a foreign gene with a retrovirus to mark the infused cells were safely transferred to patients. This study also showed that modified T cells could persist for months after transfer—a key requisite for future ACT. However, although treatment with TILs was effective in some tumours (such as melanoma), the lack of specificity and the difficulty in recruiting TILs from most other cancer types compromised the therapeutic potential.

In 1989, Zelig Eshhar and colleagues realized that the inability of T cells to recognize surface tumour antigens could be overcome by replacing domains of the T cell receptor (TCR) with antibody parts with specificity towards proteins on these cells. They combined the variable region of an antibody with the constant regions of the TCR chains, thus producing chimeric antigen receptors (CARs) that provided T cells with antibody-type specificity. The T cells expressing CARs recognized and eliminated target cells, and produced interleukin 2 in the presence of the antigen, providing a proof of concept that this approach triggers a cellular immune response.

Physiologic recognition of tumour antigens by T cells is mediated by the TCR–CD3 complex. However, this complex alone is insufficient to trigger productive T cell responses, which require the concomitant engagement of co-stimulatory receptors. In 2002, Michel Sadelain and colleagues optimized CAR design by integrating the intracellular domains of TCR and the key co-stimulatory receptor CD28 within a single molecule to help sustain T cell expansion, function and persistence. Other similar second-generation CARs subsequently emerged, incorporating different co-stimulatory domains, such as 4-1BB, which Crystal Mackall and colleagues showed can decrease the T cell exhaustion induced by continuous CAR signalling, thereby improving antitumour efficacy.

In 2010, James Kochenderfer and colleagues achieved a breakthrough with a CAR T cell therapy, reporting tumour regression in a patient with advanced follicular lymphoma, who received two infusions of autologous T cells genetically engineered to express a CAR specifically recognizing the antigen CD19 expressed on B cells. The finding that CAR T cells had activity in patients advanced the field of ACT, which saw dramatic progress in the following years and remarkable results in B-cell malignancies, including paediatric and adult acute lymphoblastic leukaemia (ALL), aggressive B cell lymphomas, chronic lymphocytic leukaemia and multiple myeloma treated with CD19 CAR T cells. In 2017, two studies—the phase II ZUMA-1 trial led by Sattva Neelapu and a case-series study led by Carl June—validated the efficacy of CD19 CAR T cells in patients with refractory B-cell leukaemia and lymphoma. Later that year, CD19 CAR T cells received US FDA approval for the treatment of children with ALL and adults with aggressive lymphomas.

Another ACT developed in parallel to CAR T cells is engineering T cells to express TCRs that recognize tumour-associated antigens. This approach offers the advantage of targeting antigens not present on the cell surface. In 2006, Rosenberg and colleagues transferred TCR T cells specifically recognizing the melanoma antigen MART-1 in 15 patients, two of whom achieved regression and still showed high levels of engineered cells in circulation one year after the infusion. This was the first clinical use of TCR T cell therapy, and although initial studies had demonstrated the feasibility and safety of introducing engineered T cells in patients, this trial also showed their remarkable efficiency. Other successful studies quickly followed, such as those demonstrating sustained complete and partial responses in patients with melanoma and treatment of synovial cell sarcoma with TCR T cells against the NY-ESO-1 antigen.

Despite the success of T cell immunotherapies, important roadblocks remain. Disease relapse and acquired resistance to CD19 CAR T cell therapy due to antigen loss can occur after initial responses. Toxic effects—such as cytokine-release syndrome or immune effector cell-associated neurotoxicity syndrome—are also an important hurdle, and biomarkers of response and toxicity are urgently needed. Novel CAR constructs are being developed to address these limitations, including multiple-antigen-targeting CARs, inclusion of cytokines to improve efficacy and conditional expression of CARs to regulate CAR activity and safety. Advances in cell engineering are enabling the use of CARs with other immune cells such as natural killer cells, and gene-editing techniques such as CRISPR-Cas9 could address limitations related to the complexity of CAR designs and the cost of manufacturing. Collectively, these new approaches could extend these revolutionary 'living drugs' to many more patients in the future.

M. Teresa Villanueva,
Nature Reviews Drug Discovery

ORIGINAL ARTICLES Maher, J. et al. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ/CD28 receptor. *Nat. Biotechnol.* **20**, 70–75 (2002) | Kochenderfer, J. N. et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood* **116**, 4099–4102 (2010) | Robbins, P. F. et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J. Clin. Oncol.* **29**, 917–924 (2011).
FURTHER READING Please visit the online article for a full list of further reading.

MILESTONE 10

Oncohistones: epigenetic drivers of cancer

Oncogenesis is often a slow process associated with the progressive accumulation of a complex network of genetic alterations, which, over time, dysregulate cellular proliferation and function. However, this aetiology is not the case for paediatric cancers—the discovery of recurrent histone mutations in children with cancer revealed that 'oncohistones' can have vast effects on gene expression and are the root causes of many aggressive paediatric cancers.

As essential components of chromatin, the core histones H2A, H2B, H3 and H4 not only create a structural backbone for eukaryotic DNA packaging, but are also crucial to the regulation of RNA replication, transcription and repair. The roles of histones in these cellular processes are regulated by their patterns of post-translational modifications, such as methylation or acetylation, and therefore depend on the activity of histone-modifying and chromatin-remodelling enzymes.

In 2012, two seminal studies revealed the presence of high-frequency somatic alterations in paediatric high-grade gliomas, including glioblastomas (GBMs). These mutations mainly affected the histone H3 genes *H3-3A* (encoding the histone variant H3.3) and *H3C2* (encoding the canonical histone H3.1). Further studies confirmed that certain paediatric tumours often contain heterozygous missense mutations in H3 genes that do not cause early protein termination and loss, but instead result in gain-of-function changes that drive oncogenesis. These amino acid substitutions often occur in the highly conserved amino terminus of H3, specifically at amino acids K27, K36 and G34, which are associated with post-translational modification of H3. These findings suggest that oncohistones promote tumour initiation and progression by interfering with the regulation of transcription through changes in chromatin remodelling and accessibility.

In 2013, multiple studies reported that the H3 K27M oncohistone leads to a decrease in trimethylated H3K27 (H3K27me3)—a transcriptional repressor—by inhibiting the methyltransferase EZH2, the catalytic subunit of Polycomb enzyme repressive complex 2 (PRC2). Interestingly, residual PRC2 activity is required for tumour proliferation, and EZH2 inhibition has therefore been proposed as a therapeutic target. The loss of H3K27me3 has been associated with the upregulation of many genes involved in developmental neurogenesis.

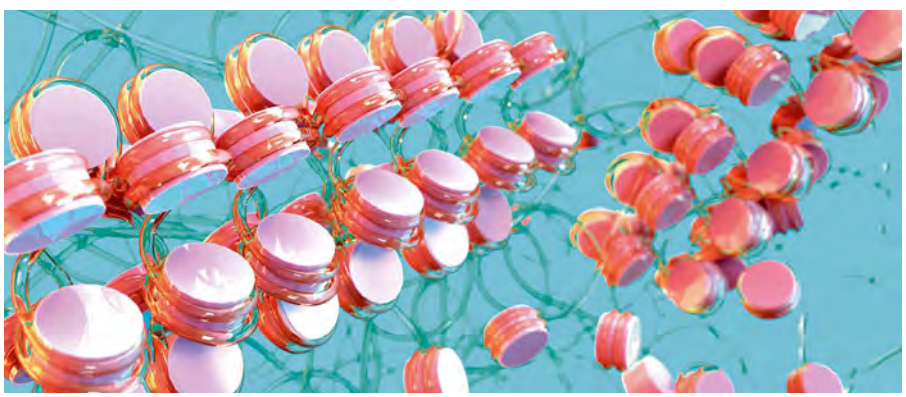
In 2012, H3 G34R substitutions were also identified as recurrent GBM alterations. A subsequent study suggested that these oncohistones alter the interaction of H3K36me3 with its binding partners, thus leading to the upregulation of the *MYCN* oncogene, and G34R inhibition of KDM4 demethylases has also been reported. Moreover, H3 K36M alterations, which have been identified in chrondroblastomas and sarcomas, can inhibit several H3K36 methyltransferases. The consequent decrease in H3K36me was associated with an increase in H3K27me, which affected the activity of PRC1 complexes and led to the expression of genes known to block mesenchymal differentiation, thus potentially contributing to sarcoma development.

Notably, these different sets of oncohistone amino acid substitutions and the genes that they affect are specifically associated with distinct tumour types and anatomical locations. For example, whereas tumours with K27 alterations often occur in the pons and thalamus, G34 alterations are usually found in tumours that develop in the cerebral hemispheres. Moreover, despite the overall decrease in methylated K27 in tumours with K27M alterations, a 2017 report suggested that some loci (such as *CDKN2A*) retain H3K27me3, thereby leading to a selective gene-silencing programme that promotes oncogenesis while retaining the identity of the tumour cell of origin. Anatomical specificity might be explained by the differential gene expression patterns of canonical and variant H3 genes across different cell types. The expression of canonical histones is restricted to the DNA-replication phase of the cell cycle, whereas histone variants can be expressed throughout any phase of the cell cycle and progressively accumulate in long-lived cells. In addition, canonical H3.1 is dispersed through the genome, whereas the H3.3 variant is incorporated into distinct genomic regions, such as areas of active transcription or regulatory regions. Interestingly, a recent study of patient-derived glioma cell lines has suggested that H3 K27M-mediated loss of H3K27me3 might occur only when oncohistones are incorporated into the chromatin of dividing cells.

A 2019 report suggested that oncohistones might not be restricted to gliomas and sarcomas. Somatic alterations in all core histones have been identified in diverse tumour types. Whether these are driver or passenger mutations remains uncertain, but the locations of the affected residues indicate that, similarly to the first-identified GBM H3 oncohistones, these mutations may have the potential to substantially override normal patterns of gene expression by interfering with post-translational histone modifications and chromatin remodelling. Further downstream, these changes are associated with alterations in kinase signalling and cellular metabolism, although the underlying mechanisms remain unclear.

These advances in understanding the roles of oncohistones in cancer have revealed new therapeutic targets, and several histone deacetylase inhibitors and tyrosine kinase inhibitors are currently being tested in clinical trials. New precision medicine efforts, which assign therapeutic interventions in clinical trials according to genetic screening of tumours, should improve the chances of success.

Monica Wang,
Nature Reviews Nephrology



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ORIGINAL ARTICLES Schwartzentruber, J. et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature* **482**, 226–231 (2012) | Wu, G. et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat. Genet.* **44**, 251–253 (2012).
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MILESTONE 11

Tumour evolution: from linear paths to branched trees

The advent of next-generation sequencing technologies enabled the characterization of cancer genomes at unprecedented resolution (MILESTONE 7). Billions of sequencing reads—representing genomes from a random subset of individual cells contained in a tumour sample—were used to build detailed catalogues of somatic mutations by comparing patients' cancer DNA to their germline DNA.

Insights into the genomic landscapes of some cancers promised to move cancer treatment towards genotype-guided approaches. However, most cancers, even highly drug-sensitive tumours with pronounced initial responses, develop resistance to targeted molecular therapies (MILESTONE 1). In 2012, Gerlinger et al. confirmed what had long been suspected: cancers are highly dynamic evolutionary entities presenting major challenges to personalized medicine. The cancer genome is characterized by extreme heterogeneity not only across tumour types, primary and secondary tumours or individuals with the same histological tumour type, but also within individual tumours.

The notion of cancer as an evolutionary process was introduced in 1976 by Peter Nowell, who hypothesized that natural selection acting on tumours in the form of clonal selection continuously drives evolutionary adaption. Nowell's original evolutionary model reflected an essentially linear path with sequentially dominant clones underlying disease progression. The idea that tumour genetic heterogeneity fuels therapeutic resistance was subsequently taken up by James Goldie and Andrew Coldman, who posited that clonal heterogeneity of tumours is the predominant factor underlying evolutionary changes resulting from cancer treatment.

In the 2000s, several studies emerged characterizing the genetic basis for disease progression, metastasis, resistance and relapse in both haematological and solid cancers. These studies demonstrated the complexity of clonal evolution and supported a model in which clonal diversity underlies disease progression and resistance to therapy.

In 2011, Anderson et al. and Notta et al. tracked the evolutionary paths taken by different subclones during progression of acute lymphoblastic leukaemia. Both studies showed that the degree of genetic heterogeneity in the leukaemia-initiating cell subpopulation was similar to that in the population of leukaemia cells in the sample. Moreover, branching evolutionary trajectories did not fit the linear clonal succession model of cancer evolution, whereby cancers progress through single-cell clone bottlenecks.

Gerlinger et al. then provided a direct demonstration of multiple genetically related subclones within a solid tumour and their phylogenetic relationships. The team applied exome sequencing, chromosome aberration analysis and ploidy profiling to primary renal carcinoma and associated metastasis samples obtained from four patients before and after treatment. Strikingly, no two samples from the same patient were genetically identical, and phylogenetic analyses revealed a branching pattern rather than linear tumour evolution. This study drove the shift in thinking of tumours in Darwinian terms—from linear to tree-like cancer evolution. The evolutionary forces of mutation, genetic drift and selection act on billions of cancer cells and their microenvironment, thus giving rise to tumours' emergent behaviours. New mutations and selection acting on mutations beneficial to the tumour drive the expansion of subclones; subsequently, cell division and continuing acquisition of mutations generate the genetic heterogeneity needed for evolutionary adaptation.

> "Mutation, genetic drift and selection give rise to a tumour's emergent behaviour"

Genomic data have enabled inferences regarding the evolutionary forces that act on cancer clones as they evolve, yielding insights into the generation of genetic variation and the order, rate and mechanisms through which this evolution occurs. Since the work by Gerlinger et al., new approaches to target cancer evolution have emerged, and focus has shifted to dissecting how subclonal populations might interact (antagonizing or synergizing) to evolve and affect clinical outcomes. Interactions between clonal driver mutations (those within the phylogenetic tree's 'trunk') and subclonal driver mutations (those within a phylogenetic branch) and competition among subclones define the clonal architecture of individual cancers. This dynamic is shaped by the pressures exerted by the environment, such as drug treatment.

Genetic heterogeneity fuels therapeutic resistance. A comprehensive understanding of the dynamics of cancer evolution and assessment of tumour heterogeneity will therefore be essential for prognostication, drug development and therapy.

Linda Koch,
Nature Reviews Genetics

ORIGINAL ARTICLE Gerlinger, M. et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N. Engl. J. Med.* **366**, 883–892 (2012).
FURTHER READING Please visit the online article for a full list of further reading.

MILESTONE 12

Undruggable? Inconceivable

In the cult-classic film *The Princess Bride*, every time the Man in Black (the good guy) overcomes an insurmountable obstacle, Vizzini (the bad guy) exclaims, "Inconceivable!" This happens repeatedly, until eventually Vizzini's companion tells him, in a moment of comedic perfection, "You keep using that word. I do not think it means what you think it means."

So it was with 'undruggable' RAS. In the early 2000s, when dozens of kinase inhibitors were heading to the clinic, comparatively little progress was being made on key non-kinase targets in oncology. RAS, a small GTPase, is one of the most frequently altered proteins in cancer and was therefore pursued with particular fervour. The lack of success in identifying RAS inhibitors led many to call it undruggable.

Credit: Lobo36

Then, in 2013, Kevin Shokat's lab developed a groundbreaking inhibitor of the KRAS G12C mutant, one of the most prevalent RAS alterations in non–small cell lung cancer (NSCLC). Shokat's compound forms a covalent bond with the inherently reactive cysteine residue at position 12 in the altered form of RAS, whereas wild-type RAS has a glycine in this position and does not react with Shokat's compound.

This discovery formed the basis of Wellspring Biosciences, which turned the compound into a more drug-like molecule, ARS-1620. From this growing field of covalent RAS inhibitors, two drugs have since been developed and shown signs of efficacy in early-stage clinical trials: Amgen's sotorasib (AMG510) and Mirati's MRTX849.

Both sotorasib and MRTX849 form covalent bonds with the reactive cysteine in KRAS-G12C.

> "In 2013, Kevin Shokat's lab developed a groundbreaking inhibitor of the KRAS-G12C mutant, one of the most prevalent RAS mutations in non-small-cell lung cancer"

In mouse models of cancers driven by KRAS-G12C, these agents induce tumour regression as monotherapies. Their anticancer activity is enhanced when they are combined with other chemotherapeutic agents (particularly those that inhibit kinases downstream of RAS) or, for sotorasib, with checkpoint inhibitors.

In the phase I trial of AMG510, no dose-limiting toxic effects or treatment-related deaths were observed. In patients with NSCLC or colorectal cancer, disease control (stable disease or an objective response) occurred in 88% or 74% respectively, of this small patient group. The extent of efficacy of MRTX849 and AMG510 will be determined in ongoing and future larger clinical trials.

RAS cycles between GTP-bound and GDP-bound states. Unexpectedly, the G12C inhibitors bind the GDP-bound, inactive state, thus demonstrating that KRAS G12C is not constitutively active (as previously thought) but is hyperexcitable. This key insight into RAS biology was enabled by the covalent inhibitors and spurred investigations into state-specific RAS-binding compounds.

Not all RAS alterations are G12C, and efforts are underway to find compounds that inhibit non-G12C RAS alterations. Different tumour types tend to have different *RAS* mutations—although nearly half the RAS alterations found in lung cancer are G12C, the G12D alteration is more prevalent in pancreatic and colon cancers.

One of the more promising pan-mutation approaches is to target the interactions between RAS and either its downstream effectors or SOS, a guanine-nucleotide-exchange factor that activates RAS. Genentech, Boehringer Ingelheim and academic researchers including Terence Rabbitts have used structure-based design to identify compounds that inhibit these interactions and thus downstream signalling. Indeed, the Boehringer Ingelheim compound also decreases the growth of NSCLC cells with *RAS* mutations.

Compounds that inhibit multiple forms of RAS could have toxic, on-target effects in non-cancerous cells. Evidence from preclinical studies, however, suggests that differences in affinity for wild-type versus mutant RAS could provide a therapeutic window for these compounds.

Compounds targeting KRAS isoforms other than G12C that are commonly found in NCSLC, pancreatic cancer and colorectal cancer are also in development. These compounds may avoid the toxicities that could arise from pan-mutation RAS inhibitors.

Efforts to find inhibitors for other non-kinase targets have had less success than those for RAS. p53, another lucrative anticancer target, remains clinically untamed. Indeed, the nutlins, a much-lauded group of potential p53-inhibitory compounds from Roche, failed in a phase III trial in acute myeloid leukaemia in mid-2020. MYC, also high on the list of cancer targets, remains undrugged.

Targeted degradation could be useful for these other undruggable proteins. Proteolysis-targeting chimeras (PROTACs) serve as synthetic E3 ligases, which bind target proteins and mark them for ubiquitin-mediated degradation. Originally conceived in Craig Crews's lab in 2001, the first PROTAC, which induces degradation of the androgen receptor, successfully completed a phase I trial in 2020, showing some signs of antitumour efficacy.

Although many non-kinase cancer targets remain undrugged, the success of covalent KRAS inhibitors and PROTACs demonstrates that developing therapeutic compounds that target these proteins may be not only conceivable but also well within the range of current possibilities.

Megan Cully,
Nature Reviews Drug Discovery

ORIGINAL ARTICLE Ostrem, J. et al. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature* **503**, 548–551 (2013).
FURTHER READING Please visit the online article for a full list of further reading.

MILESTONE 13

Good bacteria make for good cancer therapy

Credit: nobeastsofierce Science / Alamy Stock Photo

The commensal bacteria that colonize the gastrointestinal mucosa profoundly affect host physiological functions including metabolism, cellular proliferation, inflammation and immunity. These effects are driven by the ability of resident bacteria to produce small molecules and metabolites as well as to coordinate cross-talk with host immune cells both at the intestinal barrier and systemically. Many current cancer therapies rely on stimulation of antitumour immune responses, but whether the gut microbiota might influence host responses to cancer therapeutics via the immune system was largely unknown.

In 2013, two seminal studies from the groups of Laurence Zitvogel and Giorgio Trinchieri shed light on this idea. In tumour-bearing mice that lacked microbiota, either through treatment with antibiotics or using germ-free animals, the authors demonstrated that priming of both the innate and adaptive immune system by an intact gut microbiota is crucial for the effectiveness of three anticancer regimens. Iida et al. showed that the responses to CpG oligodeoxynucleotide (ODN), oxaliplatin chemotherapy and blockade of the interleukin receptor IL-10R are attenuated in tumour-bearing mice lacking gut microbiota. Mechanistically, in the case of CpG ODN, this effect is the result of decreased tumour necrosis brought about by decreased production of inflammatory cytokines from insufficiently primed tumour-infiltrating innate myeloid cells. In the case of oxaliplatin, a lack of production of reactive oxygen species by non-primed myeloid cells is partially accountable for the lower genotoxicity of the chemotherapeutic drug in the absence of a gut microbiota.

In a study by Viaud et al., naive mice treated with the chemotherapeutic drug cyclophosphamide (CTX) displayed compositional changes in the small intestinal microbiota and accompanying disruption of the intestinal barrier, thus leading to the translocation of select Gram-positive bacterial species into secondary lymphoid organs. Once in the spleen, the commensal bacteria induced a robust adaptive immune response characterized by type 17 helper T cells (T_H17) and memory type 1 helper T cells. Indeed, the therapeutic effect of CTX on tumours was mitigated in germ-free or antibiotic-treated mice, and was partly reinstated with the adoptive transfer of T_H17 cells, thus underscoring the profound effect of the microbiota in treatment outcomes.

In 2015, two studies went further and pinpointed distinct bacterial species responsible for the modulation of antitumour immune responses under therapeutic pressure. Efforts from the groups of Thomas Gajewski and Zitvogel aimed at exploring the effects of the gut microbiota specifically in the context of immune-checkpoint inhibitors (ICIs), which elicit T cell responses to tumours. Comparing genetically similar mice purchased from two different companies known to have varying compositions of commensal microbiota, Sivan et al. revealed differences in subcutaneous melanoma outgrowth that are attributable to tumour-specific T cell responses. Subsequently, *Bifidobacterium* spp. were identified by profiling the gut microbiome through sequencing of 16S ribosomal RNA and were found to be associated with both delayed tumour growth and optimal responses to an anti-PD-L1 ICI in mice. Similarly, Vétizou et al. found that the immunostimulatory activity and tumour control of CTLA-4 blockade that is decreased in germ-free or antibiotic-treated mice is restored after oral gavage with specific *Bacteroides* spp., particularly *B. fragilis*.

Until that point, most of the important initial insights into how the gut microbiota sets the inflammatory tone during therapy had come from preclinical mouse models. Consequently, growing impetus prompted researchers to evaluate whether similar observations could be made in patient cohorts. These efforts culminated in the 2018 publication of three parallel studies led by Zitvogel, Gajewski and Jennifer Wargo, which used metagenomic shotgun sequencing of patient stool samples to demonstrate that the efficacy of anti-PD-1 ICIs in people with melanoma and epithelial tumours is shaped by gut commensal bacteria.

Compositional differences in the gut microbiota were associated with clinical responses to ICIs. Importantly, reconstituting germ-free mice with faecal transplants from non-responding patients blunts the antitumour effects of PD-1 blockade by restraining local and systemic immunity, thus suggesting that the composition of the gut microbiome might be one factor accounting for primary resistance to ICIs. Unexpectedly, across these studies, little overlap was found in the specific bacterial taxa or species correlating with successful response, thus prompting the researchers to speculate on the variables that might account for these differences, such as the techniques used to analyse patient samples, geographical diversity, and diet and lifestyle factors.

Confirmation that gut microbial communities are an influential force mediating responses to a range of therapeutic modalities has raised the intriguing possibility that microbe-based therapies could eventually be implemented in clinical settings. For example, manipulating the human gut microbiota could take the form of dietary interventions and prebiotic supplementation, faecal microbiota transplantations, and administration of bacterial consortia or probiotics. However, before these possibilities can come to fruition in treating patients, more work is needed to identify and validate reliable predictive microbiome biomarkers and to fundamentally define what truly constitutes a favourable microbiome that can drive effective cancer therapy responses.

Anna Dart, *Nature Reviews Cancer*

ORIGINAL ARTICLES Iida, N. et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* **342**, 967–970 (2013) | Viaud, S. et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* **342**, 971–976 (2013).
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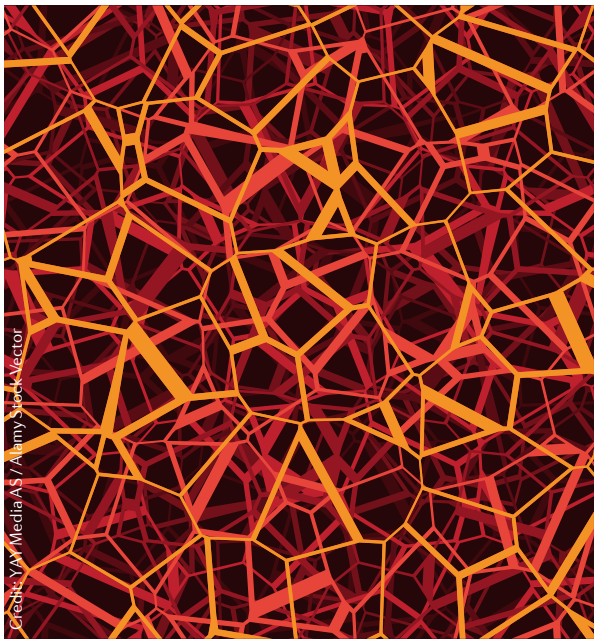
MILESTONE 14

The AI revolution in cancer

Histopathology is the current 'gold standard' for establishing cancer diagnosis. Whereas the interpretation of tissue slides is subjective, the advent of clinical genomics has contributed to refining patient stratification and clinical decision-making through the identification of actionable tumour vulnerabilities. However, both histological assessment and sequencing approaches are time consuming and costly, and they often yield inconsistent results across institutions. Enter big data and artificial intelligence (AI), with the premise that, if enough information is available, predictive models can be generated that achieve clinical-grade automated diagnoses and facilitate clinicians' workflows for accurate diagnosis.

The past decade has witnessed a surge in the amount of digitalized clinical data, including electronic health records, genomics and digital biomedical images. Perhaps because of its standardized high-quality data-collection protocols and fewer inherent missing-data challenges than in other clinical data types, medical imaging has emerged as a frontier of success for AI in medicine. Computer vision has enabled some of the most striking studies predicting molecular and pathological cues from digitalized images. The level of information that can be retrieved from digital images surpasses human capacity in terms of speed as well as the ability to infer and detect subtle traits hidden to human perception.

In 2017, Esteva et al. published a landmark study in the field of computer vision applied to cancer detection. The authors used a large dataset of digital images of skin conditions to train and validate a deep convolutional neural network able to discriminate between benign and malignant lesions with accuracy similar to that of trained dermatologists. These findings sparked further studies aimed at understanding whether additional features beyond the classification of tumour versus normal tissue—a task that can be easily conducted visually by trained pathologists—could be ascertained from histological patterns. Proof-of-concept work by Coudray et al. provided the first solid evidence that deep learning on tumour histology slides can predict driver mutations and classify tumour subtypes. Follow-up work by Campanella et al. demonstrated that high diagnostic accuracy can be achieved without a need for prior data curation, if the datasets are sufficiently large, and studies by Fu et al. and Kather et al. expanded the breadth of applicability of these algorithms in detecting molecular footprints and prognostic correlates across multiple cancers.



> The most relevant functionality of AI in cancer histopathology is its integration in clinical workflows for improving patient management.

Perhaps the most relevant functionality of AI in cancer histopathology is its integration in clinical workflows for improving patient management. For example, pioneering work by Bejnordi et al. exploring the clinical value of AI showed that deep-learning models can achieve accurate detection of lymph node metastases in breast cancer, rivalling human performance. Another pragmatic clinical application of medical AI has enabled near-real-time diagnosis of patients in the operating room. A prospective clinical trial by Hollon et al. has shown that an AI-powered system can deliver accurate diagnoses for patients undergoing surgery for brain cancer, thus underscoring the potential of these models to increase the speed and accuracy of clinical decisions.

Future studies are needed to ensure that this level of augmented perception is not available only to institutions that can afford expensive medical appliances and software. Esteva et al. paved the way to affordable access to AI-assisted differential classification of skin lesions through the use of regular cell-phone cameras. Other efforts by Chen et al. have enabled real-time integration through augmented-reality approaches which, through overlaying an AI system with a microscope field of view, facilitate automated histological annotation in places lacking access to trained pathologists. These methods have extended the real-world applications of AI a step further in the diagnosis for prostate cancer, and they may be expanded to other tumour types and diseases that often require specialized histological evaluation.

Most studies to date have been correlative and based on retrospective analysis of controlled data sets. These have provided the foundations for AI in healthcare applications and have illustrated new possibilities for enhancing the performance of trained pathologists. To fully realize their potential, AI systems must crucially be tested in controlled prospective studies to accurately evaluate their clinical value—a setting for which specific guidelines have been elaborated upon by Liu et al.

Computer vision and digital pathology are only the beginning, and AI systems are being explored in multiple additional settings. The convergence of big data and AI in oncology provides a unique interface to explore the full capabilities of these approaches for the benefit of patients. We look forward to the next 10 years of groundbreaking discoveries in this exciting area of research.

Miguel Foronda, *Nature Cancer*

ORIGINAL ARTICLE Esteva, A. et al. Dermatologist-level classification of skin cancer with deep neural networks. *Nature* **542**, 115–118 (2017).
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