Recent advances in lung cancer diagnosis: impact on patients

A report from the Roche 'Excellence in Lung Cancer Diagnosis: Impact on the Patient Pathway' meeting held in Nottingham last February, an event supported by a wide range of experts from across the UK.

According to Cancer Research UK (CRUK), lung cancer is the third most common cancer in the UK, after breast and prostate cancer, and is responsible for over 35,000 deaths per year.[1] Over the past decade, huge strides have been made in terms of survival and quality of life for lung cancer patients. We now have a better understanding of the biology of lung cancer and of the genomic aberrations that influence the pathophysiology of the disease. As a result, a number of predictive biomarkers are now used to inform appropriate treatment pathways for patients.[2]

With rapidly evolving technologies, the need for education, communication and collaboration between disciplines has never been stronger. In February 2019, Roche Diagnostics brought together key opinion-leaders and stakeholders in the area of lung cancer diagnosis and treatment to explore how the combination of new biomarkers, technological advances, targeted therapies and informatics are transforming healthcare.

Immunohistochemistry in the classification of lung cancer

There are a number of different tests that can inform a histopathology report, including:

- haematoxylin and eosin staining
- immunohistochemistry (IHC)
- *in situ* hybridisation (ISH)
- on-slide companion diagnostics (CDx).

Each test builds on the others and refines the diagnosis. A first and critical step is to establish the correct diagnosis. Immunohistochemistry can be used to great effect to improve diagnostic accuracy and, in these settings, we rely on panels of multiple markers rather than on a single IHC test. Different IHC panels can help differentiate primary lung adenocarcinoma from metastatic carcinoma, between adeno-, squamous and small cell carcinoma, and between benign mesothelial proliferation, mesothelioma and carcinoma. While this can be done using multiple IHC slides, each stained with a single antibody, we can combine several antibodies on the same slide (multiplex IHC), facilitating interpretation (Fig 1).

There is some pressure to forfeit the use of comprehensive IHC panels to save money and tissue. Concerns have been raised over the cost of multiple IHC tests. Nevertheless, diagnostic accuracy remains key, as the wrong diagnosis may result in patients being given an ineffective rather than a curative treatment – a mistake that is far more expensive than a few IHC stains. In fact, IHC is relatively low-cost when compared to all the other investigations done along the oncology patient journey, such as endoscopy and imaging.

There is also concern that IHC panels would result in excessive use of scarce tissue that is needed for other tests. The use of multiplex IHC certainly reduces the tissue requirements and we increasingly use dual-colour multiplex IHC combining two, three or even four antibodies; however, the paucity of tissue can be resolved by appropriate management of microtomy, as even a very small lung biopsy of just 0.5 mm of tissue will provide over 100 tissue sections – plenty both for all IHC required for diagnosis

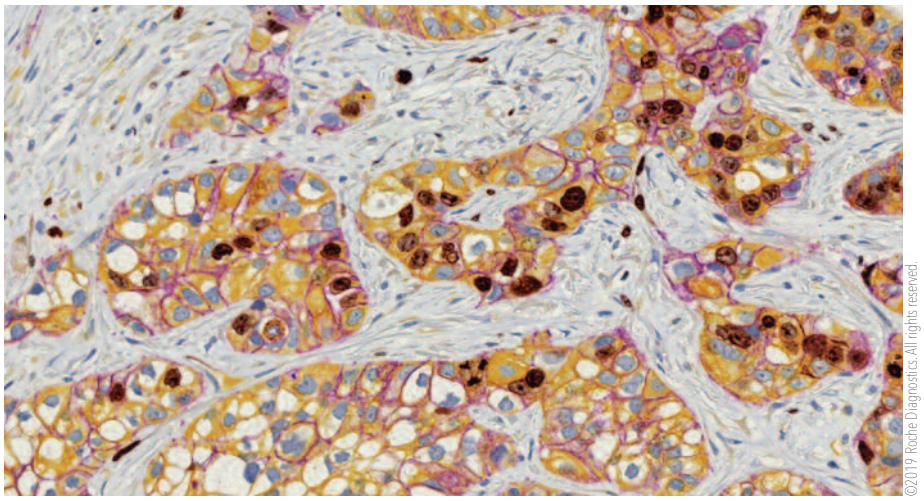


Fig 1. The use of multiple stains on a single slide.

Table 1. Molecular abnormality in lung adenocarcinoma.

Abnormality	Frequency	Possible management options
KRAS	25%	None
EGFR	23%	Erlotinib, afatinib, gefitinib, osimertinib
ALK-1	6%	Alectinib, ceritinib, crisotinib
TP53	4%	None
BRAF	3%	Debrafenib, trametinib, vemurafenib
PIK3CA	3%	None
MET	2%	Crizotinb
ROS-1	1.5%	Crizotinib, vandetanib
HER-2	1%	Ado-trastuzumab ematnsine
RET	1%	Cabozantinib, vandetanib
MEK-1	0.5%	None
NRAS	0.2%	None
β-catenin	0.2%	None
IDH-1	0.1%	None

and all subsequent tests. In future, newer chromogens with very narrow bandwidths may allow entire panels of IHC tests to be performed on a single slide, reducing further the demands on tissue.

Many cancers, including lung cancer, have a number of known molecular abnormalities that are thought to sustain cancer growth (mutation drivers) and for which there is effective targeted treatment (Table 1). Therefore, a diagnosis of cancer often requires a much more comprehensive pathology report that includes diagnostic, prognostic and predictive information to help oncologists connect patients to the right treatment. The use of multiplex assays preserves tissue that can then be used for molecular polymerase chain reaction (PCR) and next-generation sequencing (NGS) assays to help further stratify patients for treatment with targeted therapies.

EGFR testing in NSCLC

In the West Midlands, *EGFR* mutations are detected using a molecular PCR assay, including sensitising mutations (exon 19 deletions, L858R, G719X and L861Q) and resistant mutations (exon 20). Once specimens are received in the molecular laboratory, the turnaround time for the results of the PCR assays is five to seven working days, and can be down to one day if necessary.

At the Birmingham laboratory, as expected, *EGFR* mutations are found in 10.3 % of non-small cell lung cancer (NSCLC) tumours,[3] most of which (about 78%) are exon 19 deletions or L858R (Fig 2). Approximately 50% of *EGFR* mutation-positive patients who progress under tyrosine kinase inhibitor (TKI) therapy acquire an associated *EGFR* T790M mutation,[4] which conveys sensitivity to third-generation TKI therapy.

The T790M mutation is very aggressive, so there is a narrow window of opportunity to start the next treatment. This is where circulating tumour DNA (ctDNA) testing could have a role to play in identifying the T790M mutation at an earlier stage. As well as screening for the T790M mutation at the time of progression, ctDNA testing can also be performed at the time of diagnosis – if tissue biopsy is not possible – or to monitor patients on TKI therapy.

Targeted therapies for *EGFR*-positive NSCLC

Over the past decade, there has been exponential development in targeted therapeutic options in the field of lung cancer, and TKIs targeted towards *EGFR* mutations are among those approved for use in the UK.

Numerous first-line clinical trials have shown excellent response rates (around 70%) and overall survival (around two years) for TKIs compared to cytotoxic chemotherapy.[5–11] On the back of these trials, there are now three approved drugs for the treatment of EGFR-positive patients in the first-line setting: two first-generation TKIs (erlotinib and gefitinib) and a second-generation TKI (afatinib). A newer second-generation TKI (dacomitinib) has shown even better overall survival, at 34 months,[12] which has landmarked what can be achieved for *EGFR* mutation-positive patients.

No matter how well patients respond on first-line treatments, almost all will progress. Our understanding of the resistance mechanisms that affect these patients has improved. By far the most common is the acquisition of the T790M mutation, which affects 50–60% of patients progressing on a first-line EGFR-targeted TKI (Fig 3).

KEY TO SPEAKERS

Immunohistochemistry in the classification of lung cancer

Dr Corrado D'Arrigo – *Poundbury Cancer Institute, Dorchester*

***EGFR* testing in NSCLC**

Dr Philippe Taniere – *Consultant Histopathologist, Molecular Pathology, University Hospitals Birmingham NHS Foundation Trust.*

Targeted therapies for EGFR-positive NSCLC

Dr Shobhit Baijal – *Consultant Oncologist, Heart of England NHS Foundation Trust*

***ALK* and *ROS1* testing in NSCLC**

Professor Keith Kerr – *Consultant Pathologist, Aberdeen Royal Infirmary*

Targeted therapies for *ALK* gene rearrangements in NSCLC

Dr Steven Watkins – *Clinical Oncologist, University Hospitals Birmingham NHS Foundation Trust*

Routine IHC for detecting PD-L1 expression in NSCLC

Professor Manuel Salto-Tellez – *Clinical Consultant Pathologist, Queen's University Belfast*

Targeted therapies for PD-L1-positive NSCLC patients

Dr Steven Watkins – *Clinical Oncologist, University Hospitals Birmingham NHS Foundation Trust*

Next-generation sequencing as an enabling technology for clinical pathology laboratories

Professor Rachel Butler – *All Wales Medical Genetics Service*

The role of an NHS genomic hub laboratory

Dr Mike Hubank – *Head of Clinical Genomics (Research), The Royal Marsden NHS Foundation Trust*

The information explosion

Dr Matthew Prime – *Medical Director, Roche Diagnostics Information Solutions*

A third-generation TKI (osimertinib) is designed specifically to target the T790M mutation while sparing the wild-type *EGFR*. In the AURA3 clinical trial, this drug demonstrated a response rate of around 70%, with significantly improved progression-free survival compared to chemotherapy (10.1 months versus 4.4 months),[13] giving patients another highly active treatment option. Furthermore, the AURA3 team demonstrated that T790M positivity could be identified either by

ctDNA or by tissue, with virtually identical response rates (63% *vs.* 62%, respectively).[14] This may lead to establishing liquid biopsies into mainstream oncology.

ALK and *ROS1* testing in NSCLC

ALK and *ROS1* fusion genes are found in a large number of tumour types. In the context of NSCLC, they may be present in tumours where adenocarcinoma is confirmed or cannot be ruled out, and in other NSCLC patients who have never smoked or are long-time ex-smokers. Both ALK and ROS1 proteins fuse with a number of gene partners.

ALK and ROS1 are membrane-bound receptors of tyrosine kinase. In terms of their pathological activation, most recent interest has been in transphosphorylation leading to kinase activation, independent of ligand binding.[15,16]

ALK and *ROS1* fusions are relatively rare in pulmonary adenocarcinoma.[17] In Aberdeen, *ALK* fusions are present in around 3% of cases, and *ROS1* in around 1.5%. We look for them because they are excellent drug targets and ALK/ROS1-positive patients have very good response rates.[18–20]

As a result, testing for ALK and ROS1 is now embedded into CAP/IASLC/AMP guidelines, which recommend that both are included in the first tier of mandatory tests, together with *EGFR* mutation testing, in patients with adenocarcinoma.[21–23] ALK IHC is considered equivalent to *ALK* fluorescence *in situ* hybridisation (FISH) for ALK testing, and ROS1 IHC may be used as a triage assay, with positives confirmed by a molecular or cytogenetic method.

The vast majority of laboratories still rely on tissue-based testing for ALK and ROS1 by FISH or IHC. Molecular profiling by NGS can be performed in parallel. Figure 4 shows where ALK and ROS1 testing fit in the testing algorithm.

Immunohistochemistry is quick, cost-effective and readily available. However, no method is without potential pitfalls and so a potential paradigm would be to confirm ALK-positive IHC samples using FISH. ROS1-positive IHC samples already require FISH or molecular confirmation.[17]

Targeted therapies for *ALK* gene rearrangements in NSCLC

ALK gene rearrangements were first reported in a small percentage of NSCLC cases in 2007.[24] In 2011, the first targeted therapy for *ALK* rearrangements-positive NSCLC (crizotinib) received US Food and Drug Administration (FDA) approval, having demonstrated an excellent response rate (57%) and six-month progression-free survival rate (72%) in clinical trials.[25] Following further trials (first-line versus chemotherapy) in 2014,[26] this drug became a first-line treatment for *ALK* rearrangements-positive NSCLC patients.

Also in 2014, a second-generation ALK inhibitor (ceritinib) became available for second-line use in patients who progress or are intolerant to the first-line drug. In a phase 3 study, first-line use of ceritinib demonstrated improved progression-free survival compared to chemotherapy,[27] providing strong evidence for its utility as a first-line agent.

Third-generation ALK inhibitors (alectinib, brigatinib and lorlatinib [not included in the EMC and not licenced in the UK]) have also been developed and are available for second- or third-line use in patients who have progressed or are intolerant to the first-line drug. In 2017, alectinib was approved for first-line use, based on a study comparing alectinib with crizotinib in untreated ALK-positive NSCLC. This study demonstrated progression-free survival at 24 months of over 60% for patients on alectinib,[28] which is extraordinary for patients with stage 4 lung cancer. In addition, central nervous system (CNS) progression was significantly lower in the alectinib arm, which is an important consideration for patient quality of life.

Patients who progress on the approved ALK inhibitors may receive a chemotherapy doublet or immunotherapy, and future ALK inhibitors are in development. Although ALK-positive NSCLC is rare, there are several treatment options and the prognosis for patients is multiple years.

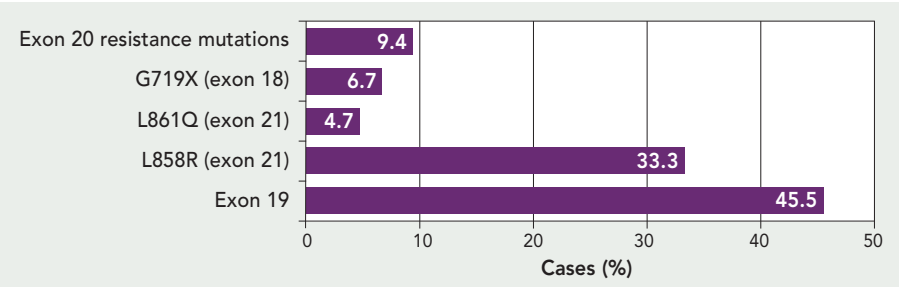


Fig 2. Spectrum of *EGFR* mutations in a series of 18,920 NSCLC tumours.

Routine IHC for detecting PD-L1 expression in NSCLC

The programmed death 1 (PD-1) pathway involves the binding of PD-1 with PD-L1 which inhibits T-cell activation, allowing immunosuppression and neoplastic growth.[29]

There are at least three PD-L1 antibodies for the analysis of PD-L1 status in clinical samples. Experience of PD-L1 IHC reflex testing using the Ventana PD-L1 SP263 assay (Roche) is described here. As a general requirement, the assay requires at least 100 tumour cells in the sample (this is not a Ventana PD-L1 SP263 assay requirement) with no strong background; there should be linear, membranous staining, which may be partial or complete (Fig 5); and only viable epithelial tumour cells are considered.

The percentage of tumour cells that are positive is recorded. If positive tumour cells are <1% of total tumour cells, the test is negative; a score of 1–49% is considered positive (low PD-L1 expression); and a score of 50% or more is considered positive (high PD-L1 expression).

SP263 assay validation showed very good equivalence to the standard 22C3 antibody.[30] Expression in adenocarcinoma and squamous cell carcinoma is very similar, and almost identical proportions of negative, low positive and high positive assays were reported in these cell types.[30] In addition, *EGFR* mutation-positive adenocarcinomas have a lower but significant level of expression,[30] which may justify reflex testing up-front in the future if the oncologist requests this information.

New approaches for PD-L1 assessment, including RNAscope and digital pathology, are becoming available and may become highly relevant in the future.

Targeted therapies for PD-L1-positive NSCLC patients

Much evidence supports the use of PD1 and PD-L1 inhibitors in the treatment of NSCLC.[31–37] A recent review of the current immunotherapy landscape for metastatic NSCLC described how five-year overall survival improved for pretreated patients on nivolumub (from <5% to 16%). This improved further to a staggering 43% for patients expressing more than 50% PD-L1,[38] which is great news for this group of patients.

The reported prevalence of PD-L1 expression in NSCLC ranges between 13–70%. In reality, approximately 40% of chemotherapy patients in my own clinics are on some form of immunotherapy. In other words, I now have a targeted treatment option for many patients who previously had a very poor prognosis.

Bearing in mind that things are changing rapidly, current lung cancer

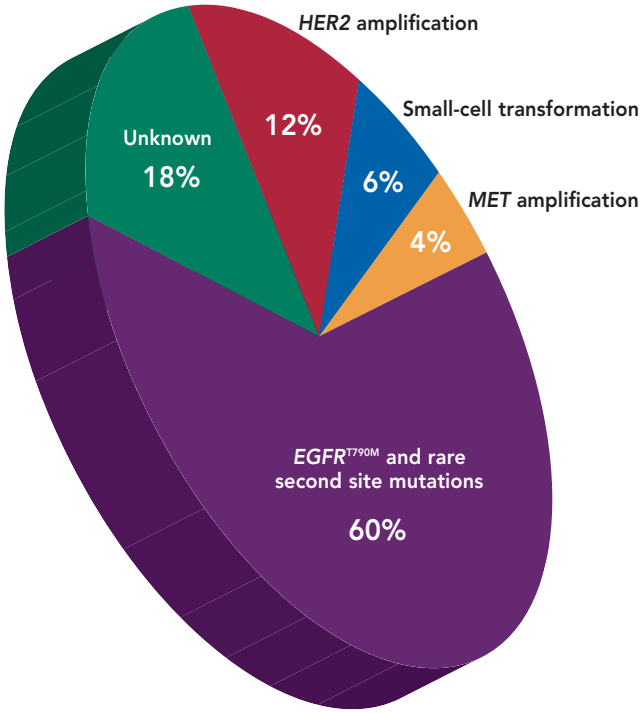


Fig 3. Acquired resistance mechanisms for EGFR-TKIs.

treatment options in the NHS directed by PDL expression are shown in Table 2. Choosing between them will often depend on the fitness of the patient and what they have received previously.

Next-generation sequencing as an enabling technology for clinical pathology laboratories

Next-generation sequencing is important for detecting certain genetic mutations and resistance mechanisms in lung cancer.[39] It covers a whole range of tests, including whole-genome sequencing (WGS) and exome sequencing, which only targets coding parts of the genome (around 5000–20,000 genes). This is of particular value in the diagnosis of rare diseases.

Alternatively, targeted gene panels (5–100 genes) can be sequenced, which is the predominant method used in oncology. The ultimate aim of targeted gene panels is to cover the genes and mutations of interest, and to be able to work with the quantity and quality of material that is provided.

There are a number of challenges for NGS in lung tumour testing, not least sample quality and quantity. The tissue provided is often formalin-fixed and paraffin wax-embedded (FFPE), which can cause random changes in the DNA sequence, and samples are usually very small. Initiatives, such as the CRUK programme, aim to improve and standardise the samples provided, which will help to improve NGS results. Education around sample standards has already made a difference.

The workflow for NGS is simple and much of it can be automated. The main steps are:

- library preparation (DNA extraction, fragmentation and hybridisation)
- cluster generation (clonal amplification to ensure detectable signal strength)
- sequencing (by synthesis, with fluorescent nucleotides)
- data analysis and reporting.

Next-generation sequencing produces a great deal of information, and bioinformatics programmes are used to sort and interpret the data. The reads are aligned against genes of interest so that recurrent variants or changes present in the sample can be detected. A list of detected variants is reported and clinicians will look to see if any are of clinical interest.

The clinical applications of NGS are widespread. In lung cancer, it is used to detect mutations that inform a diagnosis, (eg *EGFR*, *ALK* and *ROS1*). Large gene panels are beginning to be used in primary diagnostic, prognostic and clinical trial settings. Next-generation sequencing can also provide tumour signatures,[40] which can provide background information about a particular tumour. It can tell us about tumour mutation burden, which is useful in decisions about immunotherapy,[41–45] and it can detect resistance mutations, which can inform subsequent treatment decisions.[46]

Next-generation sequencing is rapidly evolving. Gene panel options are increasing, while costs and turnaround times are decreasing. In the future, NGS together with ctDNA testing may even have potential for the early detection of cancer in high-risk group patients.[47,48]

The role of an NHS genomic hub laboratory

The Royal Marsden Centre for Molecular Pathology became an NHS England regional cancer testing genomics partner (London North hub) in November 2018. There are seven such hub laboratories throughout England and, through the systematic application of genomic technologies, they aim to:[49]

- enable a quicker diagnosis for patients with rare/inherited diseases
- match people to the most effective medications and interventions
- give more accurate and early diagnosis of cancer
- facilitate more-effective use of cancer therapies.

For oncology, genomic hubs will be required to perform a range of tests from the national test directory,[50] with emphasis on panel testing to get better overall characterisation of tumours. In addition, a central facility will perform WGS, which is of value for certain cancers.

The testing portfolio will be designed to help doctors make decisions for patients, and will include targetable mutations, diagnostic and prognostic mutations, tumour burden, minimal

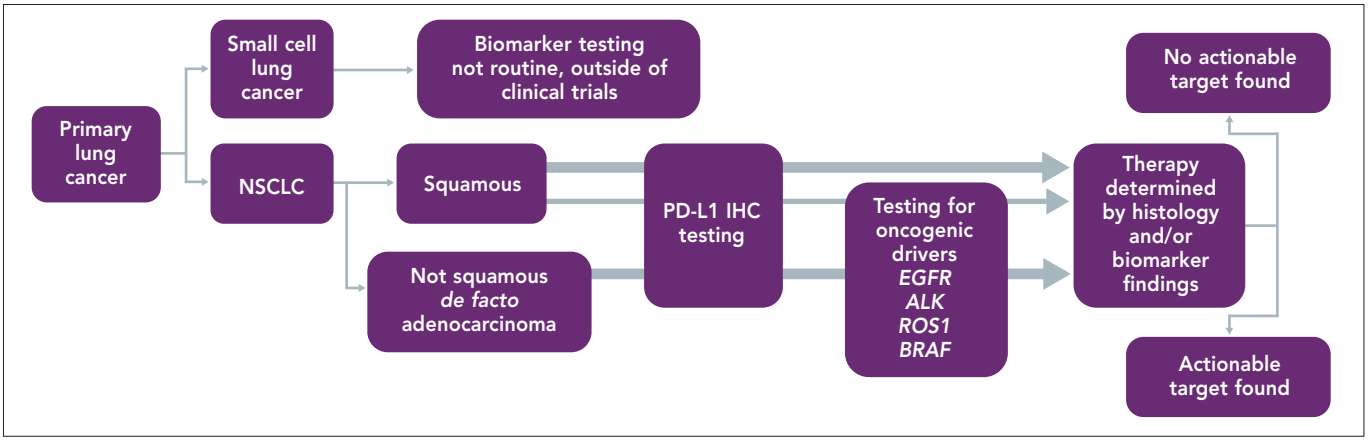


Fig 4. The lung cancer testing algorithm.

residual disease, expression classifiers (for accurate diagnosis and early detection of relapse) and immune response.

Whole-genome sequencing and exome sequencing require more time, resources, analysis and storage than a gene panel. So, if the information required can be obtained from a smaller number of genes, it makes sense to use gene panels at a fraction of the cost. A lot of effort is going into the development of clinically useful gene panels: these must be validated and verified to demonstrate their utility and performance;[51] standard operating procedures must be developed; and they must comply with quality and regulatory standards (eg MHRA, UKAS, CE-IVD and EU directives).

A wealth of information is generated by genomic tests. Much of it is actionable now, but there is more that we do not yet fully understand. A future role for specialist genomic hubs could be to collect and research those data in order to make further associations with actionable variants, and to expand that actionable portfolio to the benefit of patients.

The information explosion

The volume of medical knowledge available to us is growing at an extraordinary rate. In 1950, it is estimated that the medical knowledge doubling time was 50 years. By 1980, this estimate had increased to a doubling time of seven years, and 3.5 years by 2001. By 2020, it is projected that the doubling time for medical knowledge will be just 73 days,[52] and so it is increasingly difficult to manage and keep up with new information.

We are living in the digital information age where we are accumulating vast amounts of healthcare data every day, including that generated by new technologies such as NGS. We are also seeing fundamental changes in the healthcare landscape as our understanding of disease improves, and diagnostics and treatments become increasingly sophisticated, not least in the area of oncology. There is increasing complexity in the disease, in available diagnostics and treatments, and in workflows, which is enormously challenging.

This is where digital solutions can be of value, not only in supporting treatment decisions, but also in monitoring treatments and survivorship. Through advanced data analytics, such solutions can be used to deliver information into the hands of clinicians to inform treatment decision workflows, to enhance research and development efficiency and, ultimately, to support value-based care modules.

Tools, such as the NAVIFY Tumor Board, aim to complement human knowledge with digital knowledge. They will not replace the roles of the clinician but rather they will enhance their work and help to make maximum use of their time.

All information was correct at the time of the meeting (February 2019).

Table 2. Current lung cancer treatment options in the NHS.

Lung cancer	Treatment options
Stage IV adenocarcinoma 1st line PDL>50%	• Cis/pemetrexed + pembrolizumab • Pembrolizumab monotherapy • Nivolumab monotherapy
Stage IV adenocarcinoma 2nd line PDL >1%	If previous platinum combination: • Pembrolizumab monotherapy • Nivolumab monotherapy • Atezolizumab monotherapy
Stage IV adenocarcinoma 2nd line PDL <1%	If previous platinum combination: • Atezolizumab monotherapy
Stage IV squamous cell carcinoma 1st line PDL>50%	• Pembrolizumab monotherapy • Nivolumab monotherapy • (Cis/gemcitabine chemotherapy)
Stage IV squamous cell carcinoma 2nd line PDL <50%	If previous platinum combination: • Pembrolizumab monotherapy • Nivolumab monotherapy • Atezolizumab monotherapy
Stage IV squamous cell carcinoma PDL <1%	If previous platinum combination: • Nivolumab monotherapy • Atezolizumab monotherapy

Digital solutions can be of value, not only in supporting treatment decisions, but also in monitoring treatments and survivorship

References

1 Cancer Research UK (www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/lung-cancer).

2 Buettner R. Predictive biomarkers in lung cancer. In: Badve S, Kumar G eds. *Predictive biomarkers in oncology*. Cham, Switzerland: Springer, 2019: 403–9.

3 Evans M, O'Sullivan B, Smith M *et al*. Large-scale EGFR mutation testing in clinical practice: analysis of a series of 18,920 non-small cell lung cancer cases. *Pathol Oncol Res* 2018 Aug 9. doi: 10.1007/s12253-018-0460-2 (Epub ahead of print).

4 Stewart EL, Tan SZ, Liu G, Tsao MS. Known and putative mechanisms of resistance to EGFR targeted therapies in NSCLC patients with EGFR mutations – a review. *Transl Lung Cancer Res* 2015; **4** (1): 67–81.

5 Mok TS, Wu YL, Thongprasert S *et al*. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009; **361** (10): 947–57.

6 Han JY, Park K, Kim SW *et al*. First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. *J Clin Oncol* 2012; **30** (10): 1122–8.

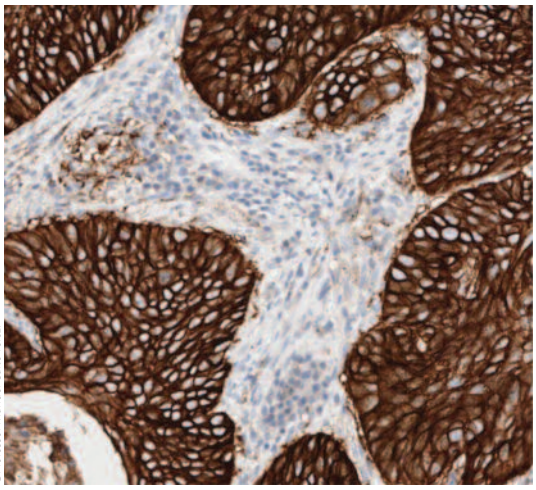
7 Maemondo M, Inoue A, Kobayashi K *et al*.; North-East Japan Study Group. Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* 2010; **362** (25): 2380–8.

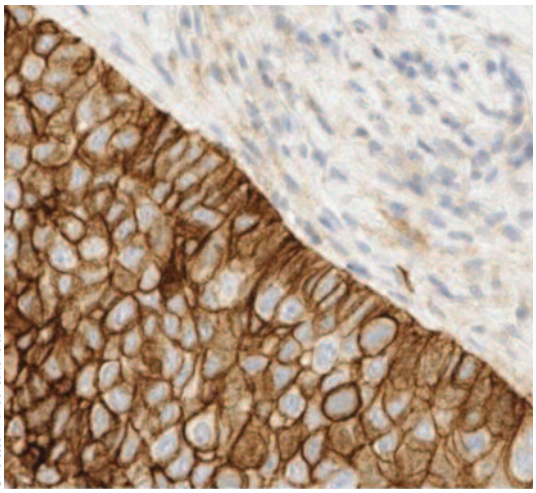
8 Mitsudomi T, Morita S, Yatabe Y *et al*.; West Japan Oncology Group. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. *Lancet Oncol* 2010; **11** (2): 121–8.

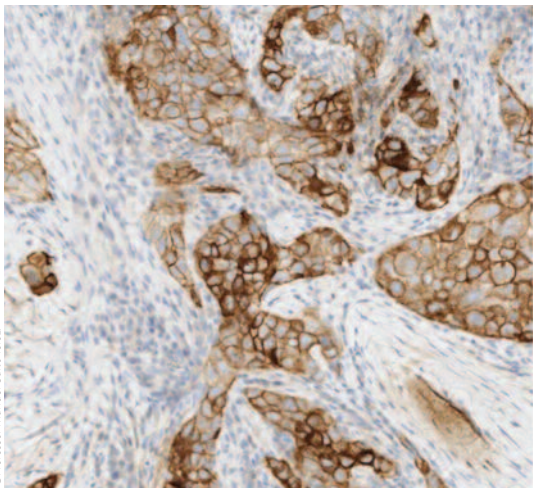
9 Herbst RS, Sun Y, Eberhardt WE *et al*. Vandetanib plus docetaxel versus docetaxel as second-line treatment for patients with advanced non-small-cell lung cancer (ZODIAC): a double-blind, randomised, phase 3 trial. *Lancet Oncol* 2010; **11** (7): 619–26.

10 Rosell R, Carcereny E, Gervais R *et al*.; Spanish Lung Cancer Group in collaboration with Groupe Français de Pneumo-Cancérologie and Associazione Italiana Oncologia Toracica. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012; **13** (3): 239–46.

11 Bai H, Wang Z, Chen K *et al*. Influence of chemotherapy on EGFR mutation status







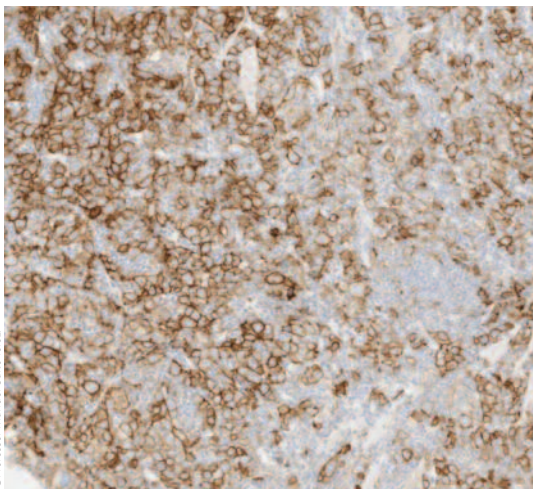


Fig 5. PD-L1-positive tumour cells.

among patients with non-small-cell lung cancer. *J Clin Oncol* 2012; **30** (25): 3077–83.
12 Mok TS, Cheng Y, Zhou X *et al*. Improvement in overall survival in a randomized study that compared dacomitinib with gefitinib in patients with advanced non-small-cell lung cancer and EGFR-activating mutations. *J Clin Oncol* 2018; **36** (22): 2244–50.
13 Mok TS, Wu Y-L, Ahn M-J *et al*. Osimertinib or platinum-pemetrexed in EGFR T790M-positive lung cancer. *N Engl J Med* 2017; **376**: 629–40.
14 Oxnard GR, Thress KS, Alden RS *et al*. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. *J Clin Oncol* 2016; **34** (28): 3375–82.
15 Hallberg B, Palmer RH. Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology. *Nat Rev Cancer* 2013; **13** (10): 685–700.
16 Lin JJ, Shaw AT. Recent advances in targeting ROS1 in lung cancer. *J Thorac Oncol* 2017; **12** (11): 1611–25.
17 Tsao MS, Hirsch FR, Yatabe Y eds. *IASLC Atlas of ALK and ROS1 Testing in Lung Cancer* 2nd edn. Aurora, Colorado: IASLC, 2016.
18 Solomon BJ, Mok T, Kim DW *et al.*; PROFILE 1014 Investigators. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med* 2014; **371** (23): 2167–77.
19 Peters S, Camidge DR, Shaw AT *et al.*; ALEX Trial Investigators. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med* 2017; **377** (9): 829–38.
20 Shaw AT, Ou SH, Bang YJ *et al*. Crizotinib in ROS1-rearranged non-small-cell lung cancer. *N Engl J Med* 2014; **371** (21): 1963–71.
21 Lindeman NI, Cagle PT, Aisner DL *et al*. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: Guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *J Thorac Oncol* 2018; **13** (3): 323–58.
22 Lindeman NI, Cagle PT, Aisner DL *et al*. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: Guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *Arch Pathol Lab Med* 2018; **142** (3): 321–46.
23 Lindeman NI, Cagle PT, Aisner D *et al*. updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: Guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *J Mol Diagn* 2018; **20** (2): 129–59
24 Soda M, Choi YL, Enomoto M *et al*. Identification of the transforming *EML4-ALK* fusion gene in non-small-cell lung cancer. *Nature* 2007; **448** (7153): 561–6.
25 Kwak EL, Bang YJ, Camidge DR *et al*. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med* 2010; **363** (18): 1693–703. Erratum in *N Engl J Med* 2011; **364** (6): 588.
26 Solomon BJ, Mok T, Kim DW *et al.*; PROFILE 1014 Investigators. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med* 2014; **371** (23): 2167–77.
27 Soria J-C, Tan DSW, Chiari R *et al*. First-line ceritinib versus platinum-based chemotherapy in advanced ALK-rearranged non-small-cell lung cancer (ASCEND-4): a randomised, open-label, phase 3 study. *Lancet* 2017; **389** (10072): 917–29.
28 Peters S, Camidge DR, Shaw AT *et al*. Alectinib versus crizotinib in untreated ALK-positive non-small-cell lung cancer. *N Engl J Med* 2017; **377** (9): 829–38.
29 Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; **12** (4): 252–64.
30 Humphries MP, McQuaid S, Craig SG *et al*. Critical appraisal of programmed death ligand 1 reflex diagnostic testing: current standards and future opportunities. *J Thorac Oncol* 2019; **14** (1): 45–53.
31 Brahmer J, Reckamp KL, Baas P *et al*. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med* 2015; **373** (2): 123–35.
32 Antonia SJ, Villegas A, Daniel D *et al.*; PACIFIC Investigators. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N Engl J Med* 2018; **379** (24): 2342–50.
33 Reck M, Rodríguez-Abreu D, Robinson AG *et al.*; KEYNOTE-024 Investigators. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016; **375** (19): 1823–33.
34 Langer CJ, Gadgeel SM, Borghaei H *et al.*; KEYNOTE-021 investigators. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *Lancet Oncol* 2016; **17** (11): 1497–508.
35 Herbst RS, Baas P, Kim DW *et al*. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016; **387** (10027): 1540–50.
36 Rittmeyer A, Barlesi F, Waterkamp D *et al.*; OAK Study Group. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* 2017; **389** (10066): 255–65.
37 Gandhi L, Rodríguez-Abreu D, Gadgeel S *et al.*; KEYNOTE-189 Investigators. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med* 2018; **378** (22): 2078–92.
38 Pabani A, Butts CA. Current landscape of immunotherapy for the treatment of metastatic non-small-cell lung cancer. *Curr Oncol* 2018; **25** (Suppl 1): S94–S102.
39 Gonzalez de Castro D, Clarke PA, Al-Lazikani B, Workman P. Personalized cancer medicine: molecular diagnostics, predictive biomarkers, and drug resistance. *Clin Pharmacol Ther* 2013; **93** (3): 252–9.
40 Wong CC, Martincorena I, Rust AG *et al*. Inactivating *CUX1* mutations promote tumorigenesis. *Nat Genet* 2014; **46** (1): 33–8.
41 Stratton MR, Campbell PJ, Futreal PA. The cancer genome. *Nature* 2009; **458** (7239): 719–24.
42 Chalmers ZR, Connelly CF, Fabrizio D *et al*. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med* 2017; **9** (1): 34.
43 Chabanon RM, Pedrero M, Lefebvre C, Marabelle A, Soria JC, Postel-Vinay S. Mutational landscape and sensitivity to immune checkpoint blockers. *Clin Cancer Res* 2016; **22** (17): 4309–21.
44 Heemskerk B, Kvistborg P, Schumacher TN. The cancer antigenome. *EMBO J* 2013; **32** (2): 194–203.
45 Giannakis M, Mu XJ, Shukla SA *et al*. Genomic correlates of immune-cell infiltrates in colorectal carcinoma. *Cell Rep* 2016; **15** (4): 857–65. Erratum in *Cell Rep* 2016; **17** (4): 1206.
46 Wagle N, Emery C, Berger MF *et al*. Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. *J Clin Oncol* 2011; **29** (22): 3085–96.
47 The Guardian. *Holy grail of cancer research: doctors positive about early detection blood test*. 1 June 2018.
48 Cohen JD, Li L, Wang Y *et al*. Detection and localization of surgically resectable cancers with a multi-analyte blood test. *Science* 2018; **359** (6378): 926–30.
49 NHS England (www.england.nhs.uk/genomics/nhs-genomic-med-service).
50 NHS England (www.england.nhs.uk/publication/national-genomic-test-directories)
51 Mattocks CJ, Morris MA, Matthijs G *et al.*; EuroGentest Validation Group. A standardized framework for the validation and verification of clinical molecular genetic tests. *Eur J Hum Genet* 2010; **18** (12): 1276–88.
52 Densen P. Challenges and opportunities facing medical education. *Trans Am Clin Climatol Assoc* 2011; **122**: 48–58.