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Tyrosine kinase gene rearrangements in epithelial malignancies

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Abstract

Chromosomal rearrangements that lead to oncogenic kinase activation are observed in many epithelial cancers. These cancers express activated fusion kinases that drive the initiation and progression of malignancy, and often have a considerable response to small-molecule kinase inhibitors, which validates these fusion kinases as ‘druggable’ targets. In this Review, we examine the aetiologic, pathogenic and clinical features that are associated with cancers harbouring oncogenic fusion kinases, including anaplastic lymphoma kinase (ALK), ROS1 and RET. We discuss the clinical outcomes with targeted therapies and explore strategies to discover additional kinases that are activated by chromosomal rearrangements in solid tumours.

Since the landmark discovery of the Philadelphia chromosome and its oncogenic product BCR–ABL in chronic myeloid leukaemia (CML), numerous other chromosomal rearrangements have been identified across different human cancers. Historically, chromosomal rearrangements have been more commonly studied in haematological rather than epithelial malignancies, in part because of the greater ease of tissue accessibility and cytogenetic analyses. However, in the past three decades the number of recurrent chromosomal rearrangements identified in common epithelial cancers has increased. Of particular interest are those rearrangements that lead to the expression of oncogenic and potentially ‘druggable’ fusion kinases. The first fusion kinases that were discovered in solid tumours involved the *RET* and neurotrophic tyrosine kinase receptor type 1 (*NTRK1*) genes in thyroid cancer¹. In 2002, the fusion of ETS variant 6 (*ETV6*; also known as *TEL*) with *NTRK3* was identified in secretory breast carcinoma, which is a rare subtype of breast cancer². Anaplastic lymphoma kinase (*ALK*) and *ROS1* fusions have more recently been found in non-small-cell lung cancer (NSCLC) and other epithelial cancers^{3,4}.

Although these cancers may have different kinase fusions, they share the common biological feature of ‘oncogene addiction’ — an increased dependency on the activated kinase for cellular proliferation and survival⁵. As a result, these cancers are often highly susceptible to small-molecule kinase inhibitors, several of which have advanced rapidly in the clinic. The discovery and successful targeting of oncogenic fusion kinases have helped to drive a major paradigm shift in oncology, whereby somatic genetic alterations — rather than the histological subtype — provide the basis for the selection of therapies.

In this Review, we focus on chromosomal rearrangements that lead to the activation of tyrosine kinases in epithelial cancers. We first discuss cellular and molecular mechanisms that may lead to chromosomal rearrangements in cancer. Then, we assess how chromosomal rearrangements can activate tyrosine kinases, how this activation leads to a state of

oncogene addiction, and how the discovery of these processes has led to new diagnostic and therapeutic opportunities in the clinic. Although this Review focuses on tyrosine kinase fusions, other kinase fusions are becoming potential drug targets (BOX 1) and might follow a similar route of development from discovery to clinical validation.

Box 1

Serine/threonine kinase rearrangements in carcinomas

Recurrent rearrangements of serine/threonine kinases, most notably RAF and microtubule-associated serine/threonine (MAST) family kinases, have recently been identified in thyroid, prostate, gastric and breast carcinomas^{25,124,125}. In particular, fusions that involve the RAF family of kinases are of considerable interest because of the recent successful development of potent RAF and MEK inhibitors in melanomas that harbour *BRAF*^{V600E} mutations.

BRAF fusions were initially identified in thyroid cancer in 2005 (REF. 25). A-kinase anchor protein 9 (*AKAP9*)–*BRAF* fusions were found in 11% of thyroid tumours that develop soon after radiation exposure. Of note, the fusions were absent in radiation-induced late-onset tumours, present in only 1% of sporadic tumours and were mutually exclusive with *BRAF* mutations. RAF family rearrangements were subsequently identified in prostate and gastric cancers¹²⁴. Gene fusions that involve ETS family transcription factors are common in prostate cancer, although they are not currently 'druggable'. However, by screening ETS rearrangement-negative prostate cancers, Palanisamy *et al.*¹²⁴ identified solute carrier family 45, member 3 (*SLC45A3*)–*BRAF* and epithelial splicing regulatory protein 1 (*ESRP1*)–*RAF1* fusions. In the study by Palanisamy *et al.*, the examination of 349 prostate cancer specimens showed six specimens with rearrangements that involved *BRAF* and four specimens with rearrangements of *RAF1*. Expression of these gene fusions resulted in the transformation of prostate cells and caused those cells to become sensitive to RAF inhibitors. By expanding the scope of their screening to include other carcinomas, the authors found a similar incidence of *BRAF* gene fusions in gastric cancer (2 out of 105). Again, those tumours that harboured the *BRAF* fusions did not contain the *BRAF*^{V600E} mutation.

Several generations of RAF and MEK inhibitors have been developed and are in various stages of clinical development¹²⁶. The RAF inhibitors vemurafenib and dabrafenib, and the MEK inhibitor trametinib, have shown clinical activity against melanomas that harbour *BRAF*^{V600E} mutations. However, it remains to be determined whether these inhibitors will have clinical efficacy against cancers that harbour RAF fusions.

Epidemiology and aetiology

Among unselected epithelial tumours, the overall rate of balanced rearrangements, which is defined as the even exchange of genetic material with no loss or duplication of DNA, has been estimated to be only 3%, whereas it is 19% in mesenchymal tumours and 29% in acute myelogenous leukaemia (AML)⁶. However, among cancers with an abnormal karyotype, the frequency of rearrangements is surprisingly similar; for example, chromosomal rearrangements that involve transcriptional regulators occur in 38% of haematological malignancies and in 44% of solid tumours that have abnormal karyotypes. Likewise, tyrosine kinase fusion genes have been identified in a similar proportion (5–7%) of haematological, mesenchymal and epithelial tumours that have abnormal karyotypes⁶. Although the fraction of epithelial tumours that have an abnormal karyotype is smaller than that of haematological malignancies, these observations suggest that gene fusion events

occur across all types of cancer, including a small but important fraction of epithelial cancers.

To date, ten different tyrosine kinases have been identified as fusion kinases in various different epithelial tumours (TABLE 1). Gene fusions that involve most of these tyrosine kinases have also been identified in non-epithelial tumours and implicated in their pathogenesis. The incidence of tyrosine kinase fusions widely varies depending on the kinase and the tumour type; for example, fusions of *RET* have been reported in more than one-third of papillary thyroid cancers in the United States⁷, but they are found in only 1% of NSCLCs^{8,9}. *ROS1* fusions are similarly rare in NSCLCs^{8,10}, but rearrangements that involve the related receptor tyrosine kinase anaplastic lymphoma kinase (*ALK*) are more common: they occur in 3–7% of NSCLCs^{8,11}. On the basis of an estimated 1.3 million new cases of NSCLC worldwide per year¹², small percentages of tyrosine kinase fusion incidence translate into relatively large numbers of patients. For example, more than 50,000 people worldwide who are diagnosed with NSCLC every year probably have an *ALK* rearrangement. This is similar to the total number of new cases of CML per year^{13,14}.

Environmental causes of gene rearrangements

The causal link between exposure to ionizing radiation and gene fusion events in cancer is well established, particularly for papillary thyroid cancer. Radiation has long been known to increase the probability of childhood thyroid cancer, and a large increase in childhood papillary thyroid cancer occurred in areas surrounding Chernobyl^{15,16}. Consistent with *in vitro* studies showing that irradiation induces *RET* translocations^{17,18}, *RET* gene rearrangements have been reported in more than 60% of individuals who developed papillary thyroid carcinomas after the Chernobyl nuclear accident^{19–24}. *NTRK1* and *BRAF* rearrangements have also been found in papillary thyroid cancers of patients after the Chernobyl nuclear accident, but they are much less common than *RET* rearrangements^{21,25}. Recently, *ALK* rearrangements have been reported in radiation-associated papillary thyroid carcinoma but not in sporadic cases where patients have not been exposed to increased levels of radiation²⁶.

For other epithelial cancers, no definite link between radiation exposure and gene fusion events has been established. There are clear links between radiation exposure and the development of certain cancers^{27–30}; for example, epidemiological studies have linked radon exposure to lung cancer. However, the potential involvement of radon in chromosomal rearrangements has only been shown in preclinical models^{31–33}. It is not known whether these or other radiation-associated tumours in patients are enriched in recurrent chromosomal rearrangements.

Several other risk factors for gene fusion events in epithelial cancers have been studied. DNA topoisomerase relaxes supercoiled DNA by cleaving and re-ligating double-stranded DNA. Topoisomerase poisons, including mitoxantrone, etoposide and doxorubicin, that are used to treat patients with cancer can lead to balanced rearrangements and to leukaemias, although there is little evidence to suggest that the rates of secondary non-haematological malignancies are increased³⁴. It has also been suggested that oxidative stress contributes to chromosomal instability³⁵. However, it is thought that cigarette smoking — the major risk factor for the development of lung cancer — does not cause a predisposition to lung cancers that are driven by tyrosine kinase fusions, because most of these cancers develop in patients who have never smoked or have a minimal history of smoking^{8–10,36–38}. Interestingly, certain gene fusion events occur at a younger age; for example, multiple case series have shown that patients with lung cancers that have *ALK*, *ROS1* or *RET* translocations are significantly younger than patients with lung cancer who do not have these gene rearrangements^{8–10,36–39}. Similarly, an association between *RET* rearrangements and

younger age has also been reported in studies of papillary thyroid cancer⁷, which include both radiation- and non-radiation-associated cases⁴⁰. In the case of radiation-induced *RET* rearrangements, the increased susceptibility of children compared to adults has been attributed to more rapid thyroid cell proliferation⁴¹.

At the molecular level, the precise mechanisms that underlie the development of chromosomal rearrangements have yet to be elucidated. The identification of recurrent rearrangements across a broad range of tumour types suggests the presence of common or shared molecular mechanisms that are not dependent on the kinase or the cell type. Primarily on the basis of studies in non-epithelial cells and cancers, it is likely that several steps are required: the generation of double-strand breaks (DSBs)^{42–47}, juxtaposition and aberrant joining of the DNA ends^{48–50}, and selection for gene rearrangements that confer a growth or survival advantage (FIG. 1a). As the first two steps have recently been reviewed⁵¹, we primarily focus our discussion on the third step.

Selection for oncogenic chromosomal translocations

For a long time, it was thought that the generation of DNA DSBs was random and that differential selection led to signature translocations. However, it is now appreciated that different tissues and cell types have nuclear dynamics and morphologies that may facilitate specific gene rearrangements; for example, *RET* and one of its fusion partners, coiled-coil domain-containing 6 (*CCDC6*), were shown to be juxtaposed (in close proximity to each other) in the nucleus in 35% of normal thyroid cells but only 6% of normal mammary epithelial cells⁵². The low frequency of *RET–CCDC6* juxtaposition in breast epithelial cells may explain the absence of this rearrangement in human breast cancers. Indeed, recent work has provided genome-wide evidence that the rearrangement frequency is related to the contact frequency^{53,54}. Nevertheless, biological selection for translocations in specific cell types is also thought to have an important role in the development of cancer; for example, in normal lymphocytes, *RET* and *CCDC6* colocalize in the nucleus to a similar extent as in thyroid cells⁵², which implies that the occurrence of *RET–CCDC6* fusion is equally probable in thyroid cells and lymphocytes. However, transgenic mice that have a *RET–CCDC6* fusion develop thyroid tumours and not lymphomas, and *RET–CCDC6* fusions have not been found in human lymphomas. These findings suggest that the oncogenicity of tyrosine kinase gene fusions may be influenced by the cellular context.

The importance of selection is also shown in a recent study of 14 breast cancer cell lines in which 77 gene fusion events were identified, 62% of which were localized to amplicons⁵⁵. Many of the gene fusions were recurrent or involved known, highly expressed oncogenic drivers. Closer examination of two separate recurrent gene fusions that involved ribosomal protein S6 kinase β 1 (*RPS6KB1*; a serine/threonine kinase downstream of mTOR) and the epidermal growth factor receptor (*EGFR*; a receptor tyrosine kinase) showed that cells that harbour these fusions were not addicted to signalling by the rearranged kinases. Of note, both gene fusions represented a minor allelic fraction of the amplified locus, the kinase domains of *RPS6KB1* and *EGFR* were absent from the fusions and the wild-type (or non-rearranged) kinases were still expressed. Thus, certain fusions — in particular, those associated with amplifications — may not represent clonally selected events and may simply be by-products or ‘passengers’ in the setting of considerable chromosomal disarray.

The biology of oncogenic fusion kinases

The tyrosine kinase fusions in epithelial cancers result from various types of chromosomal rearrangements, all of which lead to the fusion and aberrant activation of a tyrosine kinase (FIG. 1b). One of the most common types of rearrangement is interchromosomal translocation, which involves the exchange of chromosomal material between heterologous

chromosomes. Intrachromosomal rearrangements are also common, particularly paracentric inversions, which do not include the centromere. As an example, echinoderm microtubule-associated protein-like 4 (*EML4*)–*ALK* fusions in lung cancer are the result of paracentric inversions that involve the short arm of chromosome 2 (REF. 3). Pericentric inversions seem to be much less common; only one tyrosine kinase fusion — the kinesin family member 5B (*KIF5B*)–*RET* fusion in lung cancer — occurs as a result of a pericentric inversion in the centromeric region of chromosome 10 (56,57). Two other types of intrachromosomal rearrangements that lead to tyrosine kinase fusions are deletions and duplications. Examples of each rearrangement include the fused in glioblastoma (*FIG*)–*ROS1* fusion, which is caused by a very small intrachromosomal deletion in chromosome 6 (REF. 58), and the chromosome 2 open reading frame 44 (*C2orf44*)–*ALK* fusion, which is caused by a 5.2 megabase pair tandem duplication on chromosome 2 (REF. 56) (FIG. 1b).

Although the type of chromosomal rearrangement may differ, several common features define tyrosine kinase fusions in cancer. First, the portion of the tyrosine kinase gene that is involved in the fusion encodes the intact kinase domain, which includes a GXGXXG motif that is highly conserved across all the fusions and is essential for activity⁵⁹ (FIG. 2). Second, for each tyrosine kinase the breakpoint is often conserved across the various fusions; for example, for *ALK* the breakpoint is found in intron 20 across most of the fusions in lung cancer and other tumour types^{60–62}. In addition, breakpoints usually occur within one of the three introns that are located 5' to the GXGXXG-encoding exon⁵⁹ (FIG. 2). Third, each tyrosine kinase often has numerous fusion partners, even in the same disease (TABLE 1); for example, in papillary thyroid cancer there are more than ten known *RET* partners. Different portions of a partner gene may be fused to the tyrosine kinase, which generates additional 'variants' and further complicates the situation. In *ALK*-rearranged lung cancer, there are five known partners — *EML4*, TRK-fused gene (*TFG*), *KIF5B*, kinesin light chain 1 (*KLC1*) and striatin (*STRN*) — that constitute approximately 30 different fusion variants, 22 of which are *EML4*–*ALK* variants^{62,63}.

Despite the diversity of fusion partners, tyrosine kinase fusions typically share a common mechanism of kinase activation. Most fusion partners contain coiled-coil or leucine zipper domains that drive the dimerization or oligomerization of the fusion kinase, which leads to ligand-independent activation of the tyrosine kinase. Almost all *ALK* and *RET* fusion partners contribute one or more coiled-coil domains to their respective kinase fusions. Other dimerization motifs that may have an important role in kinase activation include sterile alpha motif (SAM), LisH and BAR domains, which have been recently observed in fibroblast growth factor receptor 1 (FGFR1) fusions^{64–67}.

Although oligomerization of the fusion kinase seems to be the dominant mechanism of tyrosine kinase activation, several other mechanisms have also been reported; for example, rather than providing dimerization motifs, fusion partners can provide 5 regulatory sequences that drive high-level expression and activation of tyrosine kinases. For *ALK* fusions, the endogenous *ALK* gene is normally not expressed in most adult tissues, including the lung epithelium, but rearrangement leads to both ectopic expression and constitutive activation of the *ALK* fusion protein. Kinase activation that occurs as a result of increased gene expression has been observed with several distinct fusions, which include *SLC45A3* (solute carrier family 45, member 3)–*FGFR2* in prostate cancer and SR-related C-terminal domain-associated factor 11 (*SCAF11*)–platelet-derived growth factor receptor-α (*PDGFRA*) in lung adenocarcinoma^{64,68}. In the case of the *SLC45A3*–*FGFR2* fusion, the non-coding first exon of *SLC45A3* is fused to the entire coding region of *FGFR2*, which results in considerable over-expression of the native tyrosine kinase. Other possible mechanisms of tyrosine kinase activation include increased expression resulting from loss of microRNA regulation, and conformational changes that favour the activated state^{69,70}. Interestingly, in

contrast to ALK, RET and FGFR1–FGFR3, ROS1 fusion partners frequently do not contain coiled-coil or other dimerization domains⁸. It remains unknown whether any of these alternative mechanisms lead to the activation of ROS1 and other fusion kinases that lack dimerization domains.

Numerous studies have shown that the catalytic activity of tyrosine kinase is essential for the transforming capacity of fusion kinases. However, other factors — such as altered subcellular localization — may also be important. Rearrangements of *ALK* result in loss of the ALK transmembrane domain, which leads to re-localization from the plasma membrane to the cytoplasm. Most RET fusions also lack the RET transmembrane domain, but depending on the particular fusion partner they may still reside in the plasma membrane (as occurs with nuclear receptor coactivator 4 (NCOA4)–RET)⁷¹ or relocate to different cytoplasmic compartments⁷. Such changes in subcellular localization may lead to interaction with different substrates and/or differential engagement of downstream signalling pathways that are important for cellular transformation. Changes in subcellular localization may also lead to other sequelae, as suggested by studies of FGFR3–TACC3 (transforming acidic coiled-coil-containing protein 3) in glioblastoma. Mislocalization of this fusion kinase to mitotic spindle poles causes defects in mitotic and chromosomal segregation, which lead to aneuploidy⁷². In this case, the induction of aneuploidy may cooperate with constitutive kinase activity to drive tumorigenesis.

The role of tyrosine kinase fusions as oncogenic drivers has been well established in preclinical models, which include transgenic mouse models of kinase fusion-driven cancers. In these models, aberrant activation of downstream signalling pathways promotes crucial aspects of the malignant phenotype, which include uncontrolled cellular proliferation and survival. A full discussion of the signalling networks that are activated by each tyrosine kinase fusion is beyond the scope of this Review. However, one key feature that is common to cancers harbouring many kinase fusions is the dependency of the cancer on continued signalling from the oncogenic kinase for cell growth and survival. This dependency has been termed oncogene addiction, and various hypotheses have been proposed to explain its mechanistic basis^{73,74}. In one model, the regulation of key downstream signalling pathways, such as canonical MEK–ERK and PI3K–AKT pathways, is strictly controlled by the activated tyrosine kinase (for example, ALK or ROS1)⁷⁵. Inhibition of the oncogenic kinase, by the knockdown of short hairpin RNA (shRNA) or by small-molecule tyrosine kinase inhibitors, suppresses these signalling pathways, which results in growth arrest and apoptotic cell death. In several cancers that are addicted to tyrosine kinases, such as *ALK*-rearranged lung cancer, the suppression of the MEK–ERK and PI3K–AKT pathways alters the expression levels of BCL-2 family members, leading to apoptosis. For example, suppression of the MEK pathway leads to induction of the pro-apoptotic protein BIM (also known as BCL2L11)^{76–82}, and suppression of the PI3K–AKT pathway leads to either upregulation of the pro-apoptotic protein PUMA or suppression of the anti-apoptotic protein MCL1 depending on the particular type of cancer^{83,84}. Concurrent changes are required in the expression of multiple BCL-2 family members by the suppression of both MEK–ERK and PI3K–AKT pathways to promote apoptosis. In the clinic, the induction of apoptosis seems integral to tumour response, because cancers with deficient BIM expression had worse clinical outcomes when treated with tyrosine kinase inhibitors^{81,85}.

Therapeutic strategies for fusion kinases

The phenomenon of oncogene addiction underlies the considerable antitumour activity of small-molecule tyrosine kinase inhibitors in cancers that harbour tyrosine kinase fusions. Several of these tyrosine kinase inhibitors have already shown great promise in the clinic and, in the case of lung cancers with *ALK* rearrangements, have become the standard of care.

An alternative strategy for targeting tyrosine kinase fusions is the inhibition of heat shock protein 90 (HSP90), which is a molecular chaperone that is required for proper folding and stabilization of various oncogenic proteins, including kinase fusions. Below, we review the clinical results obtained from the use of these therapeutic approaches for epithelial cancers that harbour chromosomal rearrangements.

ALK inhibitors

Crizotinib was the first small-molecule tyrosine kinase inhibitor of ALK that was tested in the clinic⁸⁶. Originally developed to target the receptor tyrosine kinase MET, crizotinib was subsequently found to inhibit several other kinases, including ALK and ROS1 (as discussed below)^{87,88}. In cell-based autophosphorylation assays, crizotinib was shown to be a potent inhibitor of both MET and ALK, with a half-maximal inhibitory concentration (IC₅₀) of 8 nM and 20 nM, respectively⁸⁹. In cell lines that expressed ALK fusions, the use of crizotinib resulted in decreased levels of phosphorylated ALK and the suppression of downstream signalling pathways, which led to inhibition of cell proliferation and induction of apoptosis. Crizotinib also showed potent antitumour activity in various different human xenograft models, which included a lymphoma model that expressed the nucleophosmin (*NPM*)–*ALK* fusion and lung cancer models that harboured the *EML4*–*ALK* fusion^{88,90,91}.

Consistent with the preclinical data, crizotinib has shown significant clinical activity in patients with advanced, *ALK*-rearranged NSCLC. During Phase I and Phase II clinical trials of crizotinib, the objective response rate (the percentage of patients with a ≥30% reduction in tumour size) was approximately 60%^{92,93}. Responses were often rapid and durable; the estimated median response duration was 49.1 weeks in the Phase I study⁹². Results from the first Phase III randomized study of crizotinib were recently reported⁹⁴. In this study, patients with advanced *ALK*-rearranged NSCLC were randomized to receive either crizotinib or standard chemotherapy as their second-line treatment. Compared to chemotherapy, the use of crizotinib resulted in an increased progression-free survival (PFS; 7.7 months versus 3 months), improved the response rate (65% compared with 19%) and improved both disease-related symptoms and quality of life. In a preliminary analysis, no difference in overall survival was seen; however, this survival analysis was probably confounded by the crossover of patients from chemotherapy to crizotinib. Furthermore, across all studies to date, the side effects of crizotinib have generally been mild and manageable, probably reflecting the limited expression of *ALK* in normal adult tissues. On the basis of its safety and efficacy, crizotinib was granted accelerated approval by the US Food and Drug Administration (FDA) in August 2011, just 4 years after the discovery of *ALK* rearrangements in NSCLC³. Results from the Phase III study have established this *ALK* inhibitor as a standard therapy for patients with advanced *ALK*-rearranged NSCLC.

The successful targeting of ALK in lung cancer has spurred the development of numerous next-generation ALK inhibitors (TABLE 2). In general, these new ALK inhibitors are more potent and selective than crizotinib; for example, CH5424802 was identified in a high-throughput screen for inhibitors of ALK⁹⁵. In cell-free enzymatic assays, CH5424802 was highly potent against ALK; it had an IC₅₀ of 1.9 nM. CH5424802 was also more selective than crizotinib, and it showed little or no inhibitory activity against other kinases, which included MET and ROS1 (REFS 95,96). In a Phase I and Phase II study that recruited patients from Japan, CH5424802 was shown to be associated with a response rate of 93.5% in crizotinib-naïve, *ALK*-rearranged NSCLC⁹⁷. Whether this remarkably high response rate was because of the increased potency of CH542802 compared with that of crizotinib is uncertain. Other factors that might have contributed to the high response rate include more rigorous diagnostic screening for *ALK* rearrangements (BOX 2) and higher drug exposures because of the Asian ethnicity of the study population⁹⁸. Nevertheless, the clinical efficacy

of this highly selective ALK inhibitor confirms a singular role for ALK in mediating oncogene addiction in *ALK*-rearranged lung cancer.

Box 2

Diagnostic assays that are used in the clinic to identify recurrent chromosomal rearrangements

Several diagnostic tests are currently used in the clinic to identify recurrent chromosomal rearrangements in solid tumours. These diagnostic assays are crucially important, as they identify those patients who will probably benefit from a targeted therapy. The most common diagnostic test is a break-apart fluorescence *in situ* hybridization (FISH) assay. This assay consists of two differently coloured fluorescent probes that hybridize to sequences on either side of the translocation breakpoint. In the presence of a chromosomal rearrangement, the probes are spatially separated, which creates a visual splitting of the two colours (see white arrows in the figure, panel **a**). On occasion, a chromosomal rearrangement can also be indicated by loss of the 5 probe and the presence of an isolated 3 signal. By contrast, the native or non-rearranged chromosome is typically observed as a fused signal. An anaplastic lymphoma kinase (*ALK*) FISH diagnostic assay was used in the Phase I and Phase II trials of crizotinib, the results of which led to accelerated approval of this drug in the United States⁸⁶; indeed, *ALK* FISH is currently the gold standard in the United States and the only US Food and Drug Administration-approved diagnostic test for the detection of *ALK* rearrangements. Similar break-apart FISH assays have also been used in early-phase studies of *ROS1*-rearranged and *RET*-rearranged lung cancers^{10,110}.

As FISH requires specialized resources and considerable expertise, other diagnostic tests for chromosomal rearrangements have been developed. In the case of *ALK*-rearranged non-small-cell lung cancer (NSCLC), immunohisto-chemistry (IHC) will probably replace FISH as the diagnostic test of choice because of its wider availability and affordability. IHC is particularly suitable for *ALK*-rearranged NSCLC because the expression of *ALK* is extremely limited in adulthood and only occurs in the lung following a chromosomal rearrangement. Several *ALK*-specific antibodies are available and show high sensitivity and specificity for detecting *ALK* expression (see the figure, panel **b**). In the Phase I study of a next-generation *ALK* inhibitor, CH5424802, *ALK* IHC was used to screen patients and *ALK* FISH was used for confirmation. In Europe, an IHC companion diagnostic is already available, which uses the rabbit monoclonal *ALK* antibody D5F3 (REF. 127). IHC might also be used in the detection of *ROS1* rearrangements; in a panel of over 500 lung tumours, IHC—using the *ROS1* D4D6 rabbit monoclonal antibody—identified nine cases with *ROS1* rearrangement, eight of which were confirmed by *ROS1* FISH¹²⁸. Larger validation studies are required before *ROS1* IHC is adopted into routine practice.

As next-generation sequencing technologies continue to improve, single-gene assays such as FISH and IHC are unlikely to remain the standard diagnostic tests for chromosomal rearrangements. Instead, targeted sequencing of all known oncogenic drivers, including those involved in chromosomal rearrangements, will probably become a standard part of the diagnostic work-up for all patients with advanced cancer.

In addition to increased potency and selectivity, next-generation ALK inhibitors have variable activity against mutant forms of ALK that are resistant to crizotinib. Therefore, several new inhibitors are specifically being tested in patients who have relapsed while receiving crizotinib therapy. To date, preliminary results have been reported for two of the next-generation ALK inhibitors — AP26113 and LDK378 (TABLE 2). A Phase I study of AP26113 showed that the preliminary response rate among patients with crizotinib-resistant disease was 75% (12 out of 16 patients)⁹⁹. Similarly, LDK378 has been associated with a high response rate (near 60%) in crizotinib-resistant, *ALK*-rearranged NSCLC, and with a prolonged median PFS¹⁰⁰. The clinical activity of next-generation ALK inhibitors is noteworthy because only one-third of crizotinib-resistant patients have resistance mutations within the tyrosine kinase domain of ALK^{82,101,102}. The ability of next-generation ALK inhibitors to overcome resistance in most patients suggests that most crizotinib-resistant tumours remain ALK-dependent and that subtherapeutic inhibition of the tyrosine kinase fusion may be an important factor that contributes to crizotinib resistance.

ROS1 inhibitors

ALK inhibitors have also shown activity against ROS1 fusions in several different experimental systems; for example, in a screen that involved more than 600 cancer cell lines, ten lines showed a considerable sensitivity to the ALK inhibitor TAE684. Although eight out of these ten sensitive cell lines were known to harbour a genetic abnormality of *ALK*, such as *ALK* rearrangement, one out of the ten cell lines was a lung cancer cell line that harboured the *SLC34A2-ROS1* fusion^{4,90}. In preclinical studies ROS1 was subsequently shown to be a target of crizotinib. In cell-based autophosphorylation assays, crizotinib was a potent inhibitor of both ALK and ROS1 to similar extents¹⁰³. In addition, in cell line models that expressed the CD74-ROS1 fusion, crizotinib inhibited cell growth and suppressed phosphorylation of ROS1, which led to downregulation of downstream signalling pathways^{10,103}.

As shown in TABLE 2, most ALK inhibitors are also ROS1 inhibitors, with the exception of CH5424802. The molecular basis for this dual inhibition is straightforward. Although ROS1 is a distinct receptor tyrosine kinase, it is closely related to ALK and to the insulin receptor family of tyrosine kinases¹⁰⁴. At the amino acid level, the tyrosine kinase domains of ALK and ROS1 are very similar — the domains have 77% identity within the ATP-binding sites. On the basis of computational modelling of the tyrosine kinase domains of ALK and ROS1 bound to crizotinib, most of the amino acid differences are not predicted to significantly affect crizotinib binding. The exception to this is a Val-to-Leu change at codon 1194, which does make direct contact with crizotinib¹⁰³. However, overall the ROS1 binding site seems to be nearly identical to that of ALK, and this observation probably explains the inhibition of ROS1 by crizotinib and other ALK inhibitors.

In the clinic, crizotinib has shown significant activity in *ROS1*-rearranged NSCLC, which is reminiscent of its activity in *ALK*-rearranged NSCLC. In the ongoing Phase I study of crizotinib, the response rate among patients with advanced *ROS1*-rearranged NSCLC was

60% (21 out of 35 patients). The median PFS has not been reached, but the probability of a continued response at 6 months was 76%¹⁰⁵. On the basis of these findings, a pivotal trial of crizotinib in advanced *ROS1*-rearranged NSCLC is being planned in Asia, and we anticipate that crizotinib will probably become a standard treatment for this molecular subtype of lung cancer. Whether crizotinib has clinical activity in other *ROS1*-rearranged cancers, such as cholangiocarcinoma and gastric cancer, is unknown. The potential role of next-generation ALK inhibitors with *ROS1* activity in *ROS1*-rearranged cancers is also unknown. As there may be common resistance mechanisms in *ALK*-rearranged and *ROS1*-rearranged NSCLC⁹⁶, inhibitors that have more potent *ROS1* activity than crizotinib may represent a therapeutic strategy to overcome resistance.

RET inhibitors

RET has long been recognized as a potential oncogenic driver in human cancer. *RET* is frequently activated by mutations in medullary thyroid cancer, in both familial and sporadic cases, and by chromosomal rearrangement in papillary thyroid cancer (TABLE 1). Although most differentiated thyroid cancers, such as papillary thyroid cancers, are potentially curable — using surgery, radioactive iodine and thyroid hormone therapy — medullary thyroid cancers have a lower cure rate. For patients with unresectable or metastatic medullary thyroid cancer, the multi-targeted tyrosine kinase inhibitors vandetanib and cabozantinib have been approved by the FDA on the basis of improvements in PFS (relative to the PFS with placebo)^{106,107}. Both of these agents inhibit RET and various other tyrosine kinases including vascular endothelial growth factor receptor 1. Other multi-kinase inhibitors with anti-RET activity, such as sunitinib and sorafenib, have also shown promising results in the treatment of medullary thyroid cancer. However, several inhibitors that do not have substantial anti-RET activity have been associated with tumour responses in both medullary and papillary thyroid carcinoma, which suggests that there are important roles for kinases other than RET that may be inhibited by these multikinase inhibitors. Interestingly, although *RET* rearrangements typically occur in one-third of papillary thyroid cancers, they were not identified in patients with advanced disease in two trials that carried out tumour genotyping^{108,109}, which suggests that *RET*-rearranged papillary thyroid cancer has a more indolent disease phenotype.

Preclinical and early clinical studies suggest that RET may be a bona fide therapeutic target in NSCLC. Several different RET fusions have been identified (TABLE 1) and demonstrate oncogenic activity *in vitro* and *in vivo*. In preclinical models, tyrosine kinase inhibitors that target RET decrease the viability of cancer cells that are transformed by the KIF5B-RET fusion protein^{8,56}. On the basis of these findings, several RET inhibitors are now used in clinical trials for *RET*-rearranged lung cancers (TABLE 3). Preliminary efficacy data have recently been reported for the use of cabozantinib in advanced, *RET*-rearranged NSCLC¹¹⁰. Among three patients who were treated with cabozantinib, two of the patients achieved a confirmed response and the remaining patient demonstrated disease stabilization for more than 31 weeks. In a separate case report, vandetanib induced a favourable response in a patient with *RET*-rearranged NSCLC¹¹¹. Although the data are limited, these initial cases suggest that *RET* rearrangement defines another oncogene addiction paradigm in NSCLC.

FGFR inhibitors

FGFR family members can be activated by point mutations, gene amplifications or chromosomal rearrangements. Since the first reports of FGFR1 and FGFR3 fusions in haematological malignancies^{112,113}, various oncogenic FGFR fusions have been discovered in solid tumours, which include glioblastoma and several epithelial cancers^{64,68,72} (TABLE 1). In both cell line and xenograft experiments, FGFR fusion kinases confer sensitivity to small-molecule FGFR inhibitors such as PD173074 and pazopanib⁶⁴. Bladder cancer cell

lines that have FGFR3 fusions seem to be more sensitive to FGFR inhibitors than cell lines that have activating FGFR3 mutations^{64,114}. Furthermore, in FGFR3–TACC3-transformed Rat1A fibroblasts, treatment using PD173074 not only inhibits cell growth but also reduces the chromosomal instability and aneuploidy that is triggered by the expression of the tyrosine kinase fusion⁷².

To date, there are no reported data on the clinical efficacy of FGFR inhibitors in patients with *FGFR*-rearranged cancers. However, several FGFR inhibitors are currently in clinical development for patients with tumours that harbour genetic alterations of FGFR, which include chromosomal rearrangements (TABLE 4).

HSP90 inhibitors

HSP90 is a highly conserved molecular chaperone that has an important role in the proper folding, stabilization and activation of numerous client proteins¹¹⁵. Several tyrosine kinase fusions are HSP90 clients, including BCR–ABL and EML4–ALK. As fusion proteins may be inherently less stable than native proteins, HSP90 inhibition might be a general strategy for treating kinase fusion-driven cancers.

ALK fusions were first identified as novel HSP90 clients in anaplastic large-cell lymphoma cells that expressed *NPM–ALK*. The association of *NPM–ALK* with HSP90 was disrupted by the HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG), which led to the destabilization and degradation of the fusion protein, decreased kinase activation and decreased downstream signalling¹¹⁶. Similarly, in lung cancer, EML4–ALK has also been shown to be an HSP90 client. The sensitivity of this ALK fusion to HSP90 inhibitors was first observed in a clinical trial of retaspimycin hydrochloride (IPI-504) in patients with advanced NSCLC¹¹⁷. Several different HSP90 inhibitors — 17-AAG, 17-DMAG (17-(demethoxy),17-dimethylaminoethylamino geldanamycin), ganetespib and AUY922 — have shown activity in cell line, xenograft and genetically engineered mouse models of *ALK*-rearranged lung cancer^{91,117–120}. The degree of sensitivity to HSP90 inhibition may depend upon the particular fusion; for example, in Ba/F3 cells, EML4–ALK variants 1 and 2 were found to be sensitive to 17-DMAG, whereas EML4–ALK variant 3 was relatively resistant to this inhibitor¹¹⁹.

In addition to ALK fusions, ROS1 and RET fusions are HSP90 client proteins. In immunoprecipitation experiments, *CCDC6–RET* fusion is associated with HSP90 and its co-chaperone CDC37 (also known as p50). Treatment of a *CCDC6–RET*-expressing thyroid cell line with 17-AAG leads to a reduction in the levels of the fusion protein¹²¹. Ganetespib has also recently been shown to inhibit the viability of the same *RET*-rearranged cell line by leading to a dose-dependent destabilization of the fusion, a decrease in MAPK signalling and induction of apoptosis¹²⁰. Ganetespib is also active in various models of *ROS1*-rearranged cancers, including the lung cancer cell line HCC78, which harbours the *SLC34A2–ROS1* fusion¹²⁰. Whether different ROS1 and RET fusions have differential sensitivity to HSP90 inhibitors is not yet known.

Among the 20 HSP90 inhibitors that are currently in clinical development, three ongoing studies include molecularly defined cohorts of patients with advanced cancers that harbour tyrosine kinase rearrangements (see Supplementary information S1 (table)). To date, the results are limited, with small numbers of patients in each trial. IPI-504 use was associated with responses in two out of three patients who had advanced, *ALK*-rearranged NSCLC. All three cases were crizotinib-naïve, and each patient received IPI-504 for approximately 7 months¹¹⁷. High response rates have also been reported with ganetespib and AUY922 in crizotinib-naïve, *ALK*-rearranged NSCLC; four out of eight responses were observed using each HSP90 inhibitor^{122,123}. For AUY922, the PFS rate at 18 weeks was 62.5%¹²³, which

suggested that there may be a moderate duration of response. The clinical activity of HSP90 inhibitors in other tyrosine kinase fusion-driven epithelial cancers has not yet been reported.

Although only preliminary data are available at present, we speculate that single-agent HSP90 inhibitors will probably not be superior to tyrosine kinase inhibitors in treatment-naïve patients, in part because of their relatively narrow therapeutic index. However, there are two specific clinical settings in which HSP90 inhibitors may have potential uses. First, patients invariably develop resistance to targeted therapies such as crizotinib, which typically occurs after 1 year of treatment. As HSP90 inhibition affects not only the fusion kinase but also other client proteins, some of which may mediate resistance, HSP90 inhibitors may be effective in patients — especially those with *ALK* resistance mutations — who have relapsed while receiving tyrosine kinase inhibitors. In preclinical studies, HSP90 inhibitors have shown potent activity in models of crizotinib-resistant, *ALK*-rearranged NSCLC that harbours *ALK* resistance mutations^{82,91,120}. In the clinic, responses have been observed in a handful of crizotinib-resistant cases: in a single patient who was treated with ganetespib¹²⁰, and in 3 out of 11 patients (27%) who were treated with AUY922 (REF. 123). Second, in either treatment-naïve or resistant settings, HSP90 inhibitors may be more useful in combination with tyrosine kinase inhibitors than as single agents. Such combinations would enable targeting of the fusion kinase in two different ways, and allow the inhibition of potential mediators of resistance. In treatment-naïve patients, the dual inhibition of the fusion kinase could potentially suppress the outgrowth of clones with secondary kinase resistance mutations. Three Phase I studies are now investigating the effects of combining HSP90 inhibitors with *ALK* tyrosine kinase inhibitors in advanced, *ALK*-rearranged NSCLC (see Supplementary information S1 (table)).

Conclusions and future directions

The recent discovery of tyrosine kinase fusion events in epithelial malignancies has not only affected the molecular classification and treatment of solid tumours but also revealed new challenges in the field. Although numerous fusion kinases have already been identified, there are probably more to be discovered, and emerging next-generation sequencing technologies will probably facilitate these breakthroughs (BOX 3). As tyrosine kinase gene rearrangements generally define rare subsets of patients with common cancers (TABLE 1), the identification of suitable patients for clinical trials that test specific targeted therapies will require prospective screening of a large number of patients. This screening will potentially need to include different tumour types. Multiplexed genetic analyses that include screening for fusion kinases will be crucial to expediting the process of target discovery and clinical validation. However, the financial costs of widespread and comprehensive genotyping will need to be addressed, in particular when no clinical benefit has yet been shown.

Box 3

Discovery of novel tyrosine kinase gene rearrangements

Non-sequencing-based approaches

Fusion kinases have been discovered by both functional studies and next-generation sequencing technologies. RET fusions were first discovered by transfecting human lymphoma DNA into NIH3T3 cells to identify the transforming oncogene¹. Echinoderm microtubule-associated protein-like 4 (*EML4*)–anaplastic lymphoma kinase (*ALK*) fusions were discovered more than 20 years later by using a similar transformation assay in which mouse 3T3 fibroblasts were infected with a retroviral cDNA expression library that was prepared using the lung adenocarcinoma sample from a patient³. At around the

same time, a phosphoproteomics-based strategy was used to define the profile of activated tyrosine kinases across multiple non-small-cell lung cancer (NSCLC) samples⁴. Novel candidate driver kinases were identified using this analysis, including ALK, ROS1, platelet-derived growth factor receptor- α (PDGFR α) and epithelial discoidin domain-containing receptor 1 (DDR1). Using this same method, in-frame rearrangements between *ROS1* and the fused in glioblastoma (*FIG*) gene were subsequently discovered in cholangiocarcinoma¹²⁹.

Using breakpoint analysis of exon array data, *EML4-ALK* fusion transcripts were discovered not only in NSCLC but also in breast and colorectal cancer samples¹³⁰. Exon arrays were also used to identify a *RET-CCDC6* (coiled-coil domain-containing 6) fusion in a patient with lung cancer without any other known oncogenic drivers¹³¹. The same *RET-CCDC6* fusion had been previously discovered in papillary thyroid cancer 25 years before the exon array study by using an NIH3T3 transformation assay¹³². Existing exon array and comparative genomic hybridization (CGH) data sets have most recently been mined to identify rearrangements, such as *CEP85L-ROS1* in angiosarcoma¹³³.

In addition, systematic analyses of large-scale cancer tissue microarrays with immunohistochemistry and fluorescence *in situ* hybridization (FISH) have shown novel kinase fusions in different cancer types.

Next-generation sequencing approaches

Whole-transcriptome or RNA sequencing is currently the predominant method used to identify novel kinase rearrangements. Indeed, this method was used to identify the first *RET* rearrangement, kinesin family member 5B (*KIF5B*)–*RET*, in lung adenocarcinoma^{57,134}. An assortment of fibroblast growth factor receptor (*FGFR*) fusions has recently been identified in various solid tumours⁶⁴, which include cholangiocarcinoma, squamous cell lung cancer, bladder cancer and others (TABLE 1). Focused kinome-centered RNA sequencing methods have identified a recurrent *FGFR3-TACC3* (transforming acidic coiled-coil-containing protein 3) fusion in squamous cell lung cancer, as well as a previously unidentified *ALK* fusion, striatin (*STRN*)–*ALK*, in a lung adenocarcinoma⁶⁹. Although transcriptome sequencing is relatively fast and allows for verification of active expression, it may fail to detect rearrangements that occur within non-coding DNA.

Genome sequencing is the most comprehensive method that can be used to identify novel rearrangements, although an enrichment step is usually used to focus analyses on the sequences of interest. *ALK* and *RET* rearrangements, for example, have been identified by next-generation genomic DNA sequencing of colorectal and NSCLC samples⁵⁶. Two previously unidentified neurotrophic tyrosine kinase receptor type 1 (*NTRK1*) gene rearrangements in NSCLC samples that lacked identifiable oncogenic drivers have also been discovered using this method¹³⁵.

A technique has been developed that takes advantage of the fact that most tyrosine kinase breakpoints in cancer occur within 200 amino acids upstream of conserved GXGXXG kinase motifs⁵⁹. In a leukaemia patient with no known gene rearrangements, this technique was used to identify a novel *CEP85L-PDGFRB* fusion¹³⁶. As cancers continue to be investigated using whole-genome and transcriptome sequencing methods, it is likely that additional kinase fusions will be discovered.

As discussed in this Review, the aetiology of chromosomal rearrangements in epithelial cancers is poorly understood. Elucidation of the molecular mechanisms that lead to these rearrangements may help to identify risk factors that can be modified or other preventive methods that can reduce the incidence of these malignancies. In addition to the oncogenic

fusions that involve tyrosine kinases, rearrangements can activate other types of kinases (BOX 1) as well as nonkinase oncogenes such as transcription factors. Given the large number of kinase inhibitors that are already available, as well as the potential to drug even ‘undruggable’ targets, it is probable that therapies targeting other oncogenic fusions will also affect the diagnosis and treatment of some epithelial cancers.

Although therapies that target tyrosine kinase fusions are highly active when used in the clinic, there is a crucial need to increase the depth and duration of the remissions that are conferred by these drugs. As multiplexed genetic analyses become more common, genetic changes that accompany chromosomal rearrangements will be identified, which will potentially reveal genetic modifiers that affect the sensitivity of tumours to targeted therapies. Furthermore, current efforts to investigate how cancers become resistant to targeted therapies will continue to provide insights into the mechanisms of resistance and may allow tracking of resistant clones that evolve under the selective pressure of targeted therapies. We believe such investigations will eventually lead to more effective therapeutic regimens for patients with cancers that harbour oncogenic fusion kinases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Takahashi M, Ritz J, Cooper GM. Activation of a novel human transforming gene, *ret*, by DNA rearrangement. *Cell*. 1985; 42:581–588. This is the first report of *RET* rearrangement that leads to oncogenic kinase activation. [PubMed: 2992805]
2. Togno C, et al. Expression of the *ETV6-NTRK3* gene fusion as a primary event in human secretory breast carcinoma. *Cancer Cell*. 2002; 2:367–376. [PubMed: 12450792]
3. Soda M, et al. Identification of the transforming *EML4-ALK* fusion gene in non-small-cell lung cancer. *Nature*. 2007; 448:561–566. This is the first report of *ALK* gene rearrangements in a subset of patients with NSCLC. [PubMed: 17625570]
4. Rikova K, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell*. 2007; 131:1190–1203. These authors used a phosphoproteomics-based strategy to discover both *ALK* and *ROS1* gene rearrangements in NSCLC. [PubMed: 18083107]
5. Weinstein IB. Cancer. addiction to oncogenes — the Achilles heal of cancer. *Science*. 2002; 297:63–64. [PubMed: 12098689]
6. Mitelman F, Johansson B, Mertens F. Fusion genes and rearranged genes as a linear function of chromosome aberrations in cancer. *Nature Genet*. 2004; 36:331–334. [PubMed: 15054488]
7. Nikiforov YE. RET/PTC rearrangement in thyroid tumors. *Endocr Pathol*. 2002; 13:3–16. This is a comprehensive review of *RET* rearrangements in papillary thyroid cancer. [PubMed: 12114746]
8. Takeuchi K, et al. RET, ROS1 and ALK fusions in lung cancer. *Nature Med*. 2012; 18:378–381. Using a histopathology-based and molecular system, the authors screened tissue microarrays that contained more than 1,500 lung cancers and identified multiple tyrosine kinase rearrangements, which included novel *ROS1* and *RET* fusions. [PubMed: 22327623]
9. Wang R, et al. RET fusions define a unique molecular and clinicopathologic subtype of non-small-cell lung cancer. *J Clin Oncol*. 2012; 30:4352–4359. This study shows that *RET* fusions define a distinct molecular subtype of NSCLC. [PubMed: 23150706]

10. Bergethson K, et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol*. 2012; 30:863–870. This study defines the clinicopathological features that are associated with *ROS1*-rearranged NSCLC, and establishes ROS1 as a target of crizotinib. [PubMed: 22215748]
11. Kris MG, et al. Identification of driver mutations in tumor specimens from 1000 patients with lung adenocarcinoma: The NCI's Lung Cancer Mutation Consortium (LCMC). *J Clin Oncol Abstr*. 2011; S29:CRA7506.
12. Bray F, Ren JS, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *Int J Cancer*. 2013; 132:1133–1145. [PubMed: 22752881]
13. Ferlay J, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010; 127:2893–2917. [PubMed: 21351269]
14. Jabbour E, Cortes JE, Ghanem H, O'Brien S, Kantarjian HM. Targeted therapy in chronic myeloid leukemia. *Expert Rev Anticancer Ther*. 2008; 8:99–110. [PubMed: 18095887]
15. Duffy BJ Jr, Fitzgerald P. J Cancer of the thyroid in children: a report of 28 cases. *J Clin Endocrinol Metab*. 1950; 10:1296–1308. [PubMed: 14794754]
16. Kazakov VS, Demidchik EP, Astakhova LN. Thyroid cancer after Chernobyl. *Nature*. 1992; 359:21–22. [PubMed: 1522879]
17. Ito T, et al. *In vitro* irradiation is able to cause *RET* oncogene rearrangement. *Cancer Res*. 1993; 53:2940–2943. [PubMed: 8319199]
18. Mizuno T, Kyoizumi S, Suzuki T, Iwamoto KS, Seyama T. Continued expression of a tissue specific activated oncogene in the early steps of radiation-induced human thyroid carcinogenesis. *Oncogene*. 1997; 15:1455–1460. [PubMed: 9333021]
19. Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H, Fagin JA. Distinct pattern of *ret* oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. *Cancer Res*. 1997; 57:1690–1694. [PubMed: 9135009]
20. Fugazzola L, et al. Oncogenic rearrangements of the *RET* protooncogene in papillary thyroid carcinomas from children exposed to the Chernobyl nuclear accident. *Cancer Res*. 1995; 55:5617–5620. [PubMed: 7585643]
21. Rabes HM, et al. Pattern of radiation-induced *RET* and *NTRK1* rearrangements in 191 post-*Chernobyl* papillary thyroid carcinomas: biological, phenotypic, and clinical implications. *Clin Cancer Res*. 2000; 6:1093–1103. [PubMed: 10741739]
22. Thomas GA, et al. High prevalence of *RET/PTC* rearrangements in Ukrainian and Belarussian post-*Chernobyl* thyroid papillary carcinomas: a strong correlation between *RET/PTC3* and the solid-follicular variant. *J Clin Endocrinol Metab*. 1999; 84:4232–4238. [PubMed: 10566678]
23. Ito T, et al. Activated *RET* oncogene in thyroid cancers of children from areas contaminated by *Chernobyl* accident. *Lancet*. 1994; 344:259. [PubMed: 7913169]
24. Klugbauer S, Lengfelder E, Demidchik EP, Rabes HM. High prevalence of *RET* rearrangement in thyroid tumors of children from Belarus after the *Chernobyl* reactor accident. *Oncogene*. 1995; 11:2459–2467. [PubMed: 8545102]
25. Ciampi R, et al. Oncogenic *AKAP9-BRAF* fusion is a novel mechanism of MAPK pathway activation in thyroid cancer. *J Clin Invest*. 2005; 115:94–101. [PubMed: 15630448]
26. Hamatani K, et al. Rearranged anaplastic lymphoma kinase (*ALK*) gene in adult-onset papillary thyroid cancer amongst atomic bomb survivors. *Thyroid*. 2012; 22:1153–1159. [PubMed: 23050789]
27. Ozasa K, et al. Studies of the mortality of atomic bomb survivors, report 14, 1950–2003: an overview of cancer and noncancer diseases. *Radiat Res*. 2012; 177:229–243. [PubMed: 22171960]
28. Gilbert ES, et al. Lung cancer after treatment for Hodgkin's disease: focus on radiation effects. *Radiat Res*. 2003; 159:161–173. [PubMed: 12537521]
29. Swerdlow AJ, et al. Risk of second malignancy after Hodgkin's disease in a collaborative British cohort: the relation to age at treatment. *J Clin Oncol*. 2000; 18:498–509. [PubMed: 10653865]
30. Rage E, et al. Risk of lung cancer mortality in relation to lung doses among French uranium miners: follow-up 1956–1999. *Radiat Res*. 2012; 177:288–297. [PubMed: 22206233]
31. Dano L, Guilly MN, Dutrillaux B, Chevillard S. Clonal evolution of a radon-induced rat lung tumor. *Cancer Genet Cytogenet*. 2001; 125:52–58. [PubMed: 11297768]

32. Dano L, et al. CGH analysis of radon-induced rat lung tumors indicates similarities with human lung cancers. *Genes Chromosom Cancer*. 2000; 29:1–8. [PubMed: 10918387]
33. Hubaux R, et al. Arsenic, asbestos and radon: emerging players in lung tumorigenesis. *Environ Health*. 2012; 11:89. [PubMed: 23173984]
34. Mistry AR, et al. DNA topoisomerase II in therapy-related acute promyelocytic leukemia. *N Engl J Med*. 2005; 352:1529–1538. [PubMed: 15829534]
35. Tsai AG, Lieber MR. Mechanisms of chromosomal rearrangement in the human genome. *BMC Genomics*. 2010; 11 (Suppl 1):S1. [PubMed: 20158866]
36. Shaw AT, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor *EML4-ALK*. *J Clin Oncol*. 2009; 27:4247–4253. [PubMed: 19667264]
37. Wong DW, et al. The *EML4-ALK* fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type *EGFR* and *KRAS*. *Cancer*. 2009; 115:1723–1733. [PubMed: 19170230]
38. Yoshida A, et al. ROS1-rearranged lung cancer: a clinicopathologic and molecular study of 15 surgical cases. *Am J Surg Pathol*. 2013; 37:554–562. [PubMed: 23426121]
39. Inamura K, et al. *EML4-ALK* lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. *Modern Pathol*. 2009; 22:508–515.
40. Bongarzone I, et al. RET/NTRK1 rearrangements in thyroid gland tumors of the papillary carcinoma family: correlation with clinicopathological features. *Clin Cancer Res*. 1998; 4:223–228. [PubMed: 9516975]
41. Saad AG, et al. Proliferative activity of human thyroid cells in various age groups and its correlation with the risk of thyroid cancer after radiation exposure. *J Clin Endocrinol Metab*. 2006; 91:2672–2677. [PubMed: 16670159]
42. Mani RS, Chinnaiyan AM. Triggers for genomic rearrangements: insights into genomic, cellular and environmental influences. *Nature Rev Genet*. 2010; 11:819–829. This review summarizes the potential aetiological factors or triggers that contribute to chromosomal rearrangements. [PubMed: 21045868]
43. Alt FW, Zhang Y, Meng FL, Guo C, Schwer B. Mechanisms of programmed DNA lesions and genomic instability in the immune system. *Cell*. 2013; 152:417–429. [PubMed: 23374339]
44. Klein IA, et al. Translocation-capture sequencing reveals the extent and nature of chromosomal rearrangements in B lymphocytes. *Cell*. 2011; 147:95–106. [PubMed: 21962510]
45. Chiarle R, et al. Genome-wide translocation sequencing reveals mechanisms of chromosome breaks and rearrangements in B cells. *Cell*. 2011; 147:107–119. [PubMed: 21962511]
46. Barlow JH, et al. Identification of early replicating fragile sites that contribute to genome instability. *Cell*. 2013; 152:620–632. [PubMed: 23352430]
47. Kim N, Jinks-Robertson S. Transcription as a source of genome instability. *Nature Rev Genet*. 2012; 13:204–214. [PubMed: 22330764]
48. Lin C, et al. Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. *Cell*. 2009; 139:1069–1083. [PubMed: 19962179]
49. Gandhi M, Medvedovic M, Stringer JR, Nikiforov YE. Interphase chromosome folding determines spatial proximity of genes participating in carcinogenic RET/PTC rearrangements. *Oncogene*. 2006; 25:2360–2366. [PubMed: 16331264]
50. Mani RS, et al. Induced chromosomal proximity and gene fusions in prostate cancer. *Science*. 2009; 326:1230. This study shows that the juxtaposition of translocation partners can be mediated by transcription factors; specifically, in prostate cancer cells the androgen receptor induces proximity of the *TMPRSS2* and *ERG* genomic loci, and in the presence of androgen and genotoxic stress it promotes DSBs and subsequent formation of the *TMPRSS2-ERG* fusion. [PubMed: 19933109]
51. Bunting SF, Nussenzweig A. End-joining, translocations and cancer. *Nature Rev Cancer*. 2013; 13:443–454. This review focuses on the factors that promote chromosomal rearrangements and, in particular, the role of non-homologous end-joining. [PubMed: 23760025]
52. Nikiforova MN, et al. Proximity of chromosomal loci that participate in radiation-induced rearrangements in human cells. *Science*. 2000; 290:138–141. This study shows that the spatial

proximity of two chromosomal loci — *RET* and *CCDC6* — facilitates the formation of a *RET-CCDC6* fusion. [PubMed: 11021799]

53. Hakim O, et al. DNA damage defines sites of recurrent chromosomal translocations in B lymphocytes. *Nature*. 2012; 484:69–74. [PubMed: 22314321]
54. Zhang Y, et al. Spatial organization of the mouse genome and its role in recurrent chromosomal translocations. *Cell*. 2012; 148:908–921. [PubMed: 22341456]
55. Kalyana-Sundaram S, et al. Gene fusions associated with recurrent amplicons represent a class of passenger aberrations in breast cancer. *Neoplasia*. 2012; 14:702–708. [PubMed: 22952423]
56. Lipson D, et al. Identification of new *ALK* and *RET* gene fusions from colorectal and lung cancer biopsies. *Nature Med*. 2012; 18:382–384. Using a next-generation sequencing assay developed by Foundation Medicine, these authors identified chromosomal rearrangements involving *ALK* and *RET* in lung and colorectal cancers. [PubMed: 22327622]
57. Kohno T, et al. *KIF5B-RET* fusions in lung adenocarcinoma. *Nature Med*. 2012; 18:375–377. [PubMed: 22327624]
58. Charest A, et al. Fusion of *FIG* to the receptor tyrosine kinase *ROS* in a glioblastoma with an interstitial del(6) (q21q21). *Genes Chromosom Cancer*. 2003; 37:58–71. [PubMed: 12661006]
59. Chmielecki J, et al. Targeted next-generation sequencing of DNA regions proximal to a conserved GXGXXG signaling motif enables systematic discovery of tyrosine kinase fusions in cancer. *Nucleic Acids Res*. 2010; 38:6985–6996. [PubMed: 20587502]
60. Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. The anaplastic lymphoma kinase in the pathogenesis of cancer. *Nature Rev Cancer*. 2008; 8:11–23. [PubMed: 18097461]
61. Hernandez L, et al. Diversity of genomic breakpoints in *TFG-ALK* translocations in anaplastic large cell chimeric lymphomas: identification of a new *TFG-ALK_{XL}* gene with transforming activity. *Am J Pathol*. 2002; 160:1487–1494. [PubMed: 11943732]
62. Ou SH, Bartlett CH, Mino-Kenudson M, Cui J, Iafrate AJ. Crizotinib for the treatment of *ALK*-rearranged non-small cell lung cancer: a success story to usher in the second decade of molecular targeted therapy in oncology. *Oncologist*. 2012; 17:1351–1375. [PubMed: 22989574]
63. To KF, et al. Detection of *ALK* rearrangement by immunohistochemistry in lung adenocarcinoma and the identification of a novel *EML4-ALK* variant. *J Thorac Oncol*. 2013; 8:883–891. [PubMed: 23625156]
64. Wu YM, et al. Identification of targetable *FGFR* gene fusions in diverse cancers. *Cancer Discov*. 2013; 3:636–647. These authors used ‘integrative sequencing’ (whole-exome sequencing, transcriptome sequencing and, as needed, low-pass genome sequencing) to discover a variety of *FGFR* fusions in diverse epithelial cancer types. [PubMed: 23558953]
65. Knight MJ, Leettola C, Gingery M, Li H, Bowie JU. A human sterile α -motif domain polymerizome. *Protein Sci*. 2011; 20:1697–1706. [PubMed: 21805519]
66. Peter BJ, et al. BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. *Science*. 2004; 303:495–499. [PubMed: 14645856]
67. Mateja A, Cierpicki T, Paduch M, Derewenda ZS, Otlewski J. The dimerization mechanism of *LIS1* and its implication for proteins containing the LisH motif. *J Mol Biol*. 2006; 357:621–631. [PubMed: 16445939]
68. Seo JS, et al. The transcriptional landscape and mutational profile of lung adenocarcinoma. *Genome Res*. 2012; 22:2109–2119. [PubMed: 22975805]
69. Majewski IJ, et al. Identification of recurrent *FGFR3* fusion genes in lung cancer through kinome-centered RNA sequencing. *J Pathol*. 2013; 230:270–276. [PubMed: 23661334]
70. Parker BC, et al. The tumorigenic *FGFR3-TACC3* gene fusion escapes miR-99a regulation in glioblastoma. *J Clin Invest*. 2013; 123:855–865. [PubMed: 23298836]
71. Monaco C, et al. The RFG oligomerization domain mediates kinase activation and re-localization of the *RET/PTC3* oncoprotein to the plasma membrane. *Oncogene*. 2001; 20:599–608. [PubMed: 11313992]
72. Singh D, et al. Transforming fusions of *FGFR* and *TACC* genes in human glioblastoma. *Science*. 2012; 337:1231–1235. [PubMed: 22837387]

73. Sharma SV, Settleman J. Oncogene addiction: setting the stage for molecularly targeted cancer therapy. *Genes Dev.* 2007; 21:3214–3231. This review examines the molecular mechanisms that underlie the phenomenon of oncogene addiction. [PubMed: 18079171]
74. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell.* 2009; 136:823–837. [PubMed: 19269363]
75. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nature Rev Cancer.* 2009; 9:550–562. [PubMed: 19629070]
76. Ley R, Balmanno K, Hadfield K, Weston C, Cook SJ. Activation of the ERK1/2 signaling pathway promotes phosphorylation and proteasome-dependent degradation of the BH3-only protein, Bim. *J Biol Chem.* 2003; 278:18811–18816. [PubMed: 12646560]
77. Costa DB, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. *PLoS Med.* 2007; 4:1669–1679. discussion 1680. [PubMed: 17973572]
78. Cragg MS, Kuroda J, Puthalakath H, Huang DC, Strasser A. Gefitinib-induced killing of NSCLC cell lines expressing mutant *EGFR* requires BIM and can be enhanced by BH3 mimetics. *PLoS Med.* 2007; 4:1681–1689. [PubMed: 17973573]
79. Rahmani M, et al. The BH3-only protein Bim plays a critical role in leukemia cell death triggered by concomitant inhibition of the PI3K/Akt and MEK/ERK1/2 pathways. *Blood.* 2009; 114:4507–4516. [PubMed: 19773546]
80. Takezawa K, Okamoto I, Nishio K, Janne PA, Nakagawa K. Role of ERK-BIM and STAT3-survivin signaling pathways in ALK inhibitor-induced apoptosis in EML4-ALK-positive lung cancer. *Clin Cancer Res.* 2011; 17:2140–2148. [PubMed: 21415216]
81. Faber AC, et al. BIM expression in treatment-naïve cancers predicts responsiveness to kinase inhibitors. *Cancer Discov.* 2011; 1:352–365. [PubMed: 22145099]
82. Katayama R, et al. Mechanisms of acquired crizotinib resistance in ALK-rearranged lung cancers. *Sci Transl Med.* 2012; 4:120ra17.
83. Bean GR, et al. PUMA and BIM are required for oncogene inactivation-induced apoptosis. *Sci Signal.* 2013; 6:ra20. [PubMed: 23532334]
84. Faber AC, et al. Differential induction of apoptosis in HER2 and EGFR addicted cancers following PI3K inhibition. *Proc Natl Acad Sci USA.* 2009; 106:19503–19508. [PubMed: 19850869]
85. Ng KP, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. *Nature Med.* 2012; 18:521–528. [PubMed: 22426421]
86. Kwak EL, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N Engl J Med.* 2010; 363:1693–1703. This landmark Phase I study established crizotinib as a highly effective and safe therapy for patients with advanced, *ALK*-rearranged NSCLC. [PubMed: 20979469]
87. Zou HY, et al. An orally available small-molecule inhibitor of c-Met, PF-2341066, exhibits cytoreductive antitumor efficacy through antiproliferative and antiangiogenic mechanisms. *Cancer Res.* 2007; 67:4408–4417. [PubMed: 17483355]
88. Christensen JG, et al. Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. *Mol Cancer Ther.* 2007; 6:3314–3322. [PubMed: 18089725]
89. Cui JJ, et al. Structure based drug design of crizotinib (PF-02341066), a potent and selective dual inhibitor of mesenchymal-epithelial transition factor (c-MET) kinase and anaplastic lymphoma kinase (ALK). *J Med Chem.* 2011; 54:6342–6363. [PubMed: 21812414]
90. McDermott U, et al. Genomic alterations of anaplastic lymphoma kinase may sensitize tumors to anaplastic lymphoma kinase inhibitors. *Cancer Res.* 2008; 68:3389–3395. [PubMed: 18451166]
91. Katayama R, et al. Therapeutic strategies to overcome crizotinib resistance in non-small cell lung cancers harboring the fusion oncogene EML4-ALK. *Proc Natl Acad Sci USA.* 2011; 108:7535–7540. [PubMed: 21502504]
92. Camidge DR, et al. Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. *Lancet Oncol.* 2012; 13:1011–1019. [PubMed: 22954507]

93. Kim DW, et al. Results of a global phase II study with crizotinib in advanced ALK-positive non-small cell lung cancer (NSCLC). *J Clin Oncol.* 2012; 30 (Suppl):7533.
94. Shaw AT, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. *N Engl J Med.* 2013; 368:2385–2394. This is the first randomized study that compares crizotinib with chemotherapy in *ALK*-rearranged NSCLC. [PubMed: 23724913]
95. Sakamoto H, et al. CH5424802, a selective ALK inhibitor capable of blocking the resistant gatekeeper mutant. *Cancer Cell.* 2011; 19:679–690. [PubMed: 21575866]
96. Awad MM, et al. Acquired resistance to crizotinib from a mutation in CD74-ROS1. *N Engl J Med.* 2013; 368:2395–2401. [PubMed: 23724914]
97. Seto T, et al. CH5424802 (RO5424802) for patients with ALK-rearranged advanced non-small-cell lung cancer (AF-001JP study): a single-arm, open-label, phase 1–2 study. *Lancet Oncol.* 2013; 14:590–598. [PubMed: 23639470]
98. Ou SH, et al. Comparison of crizotinib (PF-02341066) pharmacokinetics between Asian and non-Asian patients with advanced malignancies. *J Thorac Oncol.* 2010; 5:S382.
99. Camidge DR, et al. First-in-human dose-finding study of the ALK/EGFR inhibitor AP26113 in patients with advanced malignancies: updated results. *J Clin Oncol Abstr.* 2013; 31:8031.
100. Shaw AT, et al. Clinical activity of the ALK inhibitor LDK378 in advanced, ALK-positive NSCLC. *J Clin Oncol Abstr.* 2013; 31:8010.
101. Doebele RC, et al. Mechanisms of resistance to crizotinib in patients with *ALK* gene rearranged non-small cell lung cancer. *Clin Cancer Res.* 2012; 18:1472–1482. [PubMed: 22235099]
102. Gainor JF, et al. ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. *Clin Cancer Res.* 2013; 19:4273–4281. [PubMed: 23729361]
103. Shaw AT, et al. Clinical activity of crizotinib in advanced non-small cell lung cancer (NSCLC) harboring ROS1 gene rearrangement. *J Clin Oncol Abstr.* 2012; 30:7508.
104. Acquaviva J, Wong R, Charest A. The multifaceted roles of the receptor tyrosine kinase ROS in development and cancer. *Biochim Biophys Acta.* 2009; 1795:37–52. [PubMed: 18778756]
105. Ou SH, et al. Efficacy and safety of crizotinib in patients with advanced *ROS1*-rearranged non-small cell lung cancer (NSCLC). *J Clin Oncol Abstr.* 2013; 31:8032.
106. Wells SA, et al. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. *J Clin Oncol.* 2012; 30:134–141. [PubMed: 22025146]
107. Schoffski P, et al. An international, double-blind, randomized, placebo-controlled phase III trial (EXAM) of cabozantinib (XL184) in medullary thyroid carcinoma (MTC) patients (pts) with documented RECIST progression at baseline. *J Clin Oncol.* 2012; 30 (Suppl):5508.
108. Sherman SI, et al. Motesanib diphosphate in progressive differentiated thyroid cancer. *N Engl J Med.* 2008; 359:31–42. [PubMed: 18596272]
109. Kloos RT, et al. Phase II trial of sorafenib in metastatic thyroid cancer. *J Clin Oncol.* 2009; 27:1675–1684. [PubMed: 19255327]
110. Drilon A, et al. Response to cabozantinib in patients with RET fusion-positive lung adenocarcinomas. *Cancer Discov.* 2013; 3:630–635. This is the first paper to show clinical activity of a RET inhibitor in patients with *RET*-rearranged NSCLC, validating RET as a molecular target in lung cancer. [PubMed: 23533264]
111. Gautschi O, et al. A patient with lung adenocarcinoma and RET fusion treated with vandetanib. *J Thorac Oncol.* 2013; 8:e43–e44. [PubMed: 23584301]
112. Xiao S, et al. FGFR1 is fused with a novel zinc-finger gene, ZNF198, in the t(8;13) leukaemia/lymphoma syndrome. *Nature Genet.* 1998; 18:84–87. [PubMed: 9425908]
113. Yagasaki F, et al. Fusion of ETV6 to fibroblast growth factor receptor 3 in peripheral T-cell lymphoma with a t(4;12)(p16;p13) chromosomal translocation. *Cancer Res.* 2001; 61:8371–8374. [PubMed: 11731410]
114. Williams SV, Hurst CD, Knowles MA. Oncogenic FGFR3 gene fusions in bladder cancer. *Hum Mol Genet.* 2013; 22:795–803. [PubMed: 23175443]

115. Whitesell L, Lindquist SL. HSP90 and the chaperoning of cancer. *Nature Rev Cancer*. 2005; 5:761–772. This is a comprehensive review of HSP90, and it includes a discussion of the potential therapeutic value of targeting HSP90 in cancer. [PubMed: 16175177]
116. Bonvini P, Gastaldi T, Falini B, Rosolen A. Nucleophosmin-anaplastic lymphoma kinase (NPM-ALK), a novel Hsp90-client tyrosine kinase: down-regulation of NPM-ALK expression and tyrosine phosphorylation in ALK⁺ CD30⁺ lymphoma cells by the Hsp90 antagonist 17-allylamino, 17-demethoxygeldanamycin. *Cancer Res*. 2002; 62:1559–1566. [PubMed: 11888936]
117. Sequist LV, et al. Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. *J Clin Oncol*. 2010; 28:4953–4960. [PubMed: 20940188]
118. Chen Z, et al. Inhibition of ALK, PI3K/MEK, and HSP90 in murine lung adenocarcinoma induced by *EML4-ALK* fusion oncogene. *Cancer Res*. 2010; 70:9827–9836. [PubMed: 20952506]
119. Heuckmann JM, et al. Differential protein stability and ALK inhibitor sensitivity of EML4-ALK fusion variants. *Clin Cancer Res*. 2012; 18:4682–4690. [PubMed: 22912387]
120. Sang J, et al. Targeted inhibition of the molecular chaperone Hsp90 overcomes ALK inhibitor resistance in non-small cell lung cancer. *Cancer Discov*. 2013; 3:430–443. [PubMed: 23533265]
121. Marsee DK, et al. Inhibition of heat shock protein 90, a novel RET/PTC1-associated protein, increases radioiodide accumulation in thyroid cells. *J Biol Chem*. 2004; 279:43990–43997. [PubMed: 15302866]
122. Socinski MA, et al. A multicenter Phase II study of ganetespib monotherapy in patients with genotypically defined advanced non-small cell lung cancer. *Clin Cancer Res*. 2013; 19:3068–3077. [PubMed: 23553849]
123. Felip E, et al. Phase II activity of the hsp90 inhibitor AUY922 in patients with ALK-rearranged (ALK+) or EGFR-mutated advanced non-small cell lung cancer (NSCLC). *Ann Oncol*. 2012; 23 (Suppl 9):4380.
124. Palanisamy N, et al. Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma. *Nature Med*. 2010; 16:793–798. [PubMed: 20526349]
125. Stephens PJ, et al. Complex landscapes of somatic rearrangement in human breast cancer genomes. *Nature*. 2009; 462:1005–1010. [PubMed: 20033038]
126. Huang T, Karsy M, Zhuge J, Zhong M, Liu D. B-Raf and the inhibitors: from bench to bedside. *J Hematol Oncol*. 2013; 6:30. [PubMed: 23617957]
127. Mino-Kenudson M, et al. A novel, highly sensitive antibody allows for the routine detection of ALK-rearranged lung adenocarcinomas by standard immunohistochemistry. *Clin Cancer Res*. 2010; 16:1561–1571. [PubMed: 20179225]
128. Rimkunas VM, et al. Analysis of receptor tyrosine kinase ROS1-positive tumors in non-small cell lung cancer: identification of a FIG-ROS1 fusion. *Clin Cancer Res*. 2012; 18:4449–4457. [PubMed: 22661537]
129. Gu TL, et al. Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. *PLoS ONE*. 2011; 6:e15640. [PubMed: 21253578]
130. Lin E, et al. Exon array profiling detects EML4-ALK fusion in breast, colorectal, and non-small cell lung cancers. *Mol Cancer Res*. 2009; 7:1466–1476. [PubMed: 19737969]
131. Li F, et al. Identification of RET gene fusion by exon array analyses in “pan-negative” lung cancer from never smokers. *Cell Res*. 2012; 22:928–931. [PubMed: 22349463]
132. Fusco A, et al. A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. *Nature*. 1987; 328:170–172. [PubMed: 3600795]
133. Giacomini CP, et al. Breakpoint analysis of transcriptional and genomic profiles uncovers novel gene fusions spanning multiple human cancer types. *PLoS Genet*. 2013; 9:e1003464. In this paper, by examining the transcript level or genomic DNA copy number transitions that occur within genes, the authors discover multiple gene fusions, which include a novel *ROS1* fusion in angiosarcoma. [PubMed: 23637631]

134. Ju YS, et al. A transforming *KIF5B* and *RET* gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. *Genome Res.* 2012; 22:436–445. [PubMed: 22194472]
135. Doebele RC, et al. NTRK1 gene fusions as a novel oncogenic target in lung cancer. *J Clin Oncol Abstr.* 2013; 31:8023.
136. Chmielecki J, et al. Systematic screen for tyrosine kinase rearrangements identifies a novel *C6orf204-PDGFRB* fusion in a patient with recurrent T-ALL and an associated myeloproliferative neoplasm. *Genes Chromosomes Cancer.* 2012; 51:54–65. [PubMed: 21938754]
137. Takeuchi K, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. *Clin Cancer Res.* 2009; 15:3143–3149. [PubMed: 19383809]
138. Wong DW, et al. A novel KIF5B-ALK variant in nonsmall cell lung cancer. *Cancer.* 2011; 117:2709–2718. [PubMed: 21656749]
139. Togashi Y, et al. KLC1-ALK: a novel fusion in lung cancer identified using a formalin-fixed paraffin-embedded tissue only. *PloS ONE.* 2012; 7:e31323. [PubMed: 22347464]
140. Weickhardt AJ, et al. ALK and ROS1 gene rearrangements detected in colorectal cancer (CRC) by fluorescence in situ hybridization (FISH). *J Clin Oncol Abstr.* 2013; 31:3545.
141. Jazii FR, et al. Identification of squamous cell carcinoma associated proteins by proteomics and loss of beta tropomyosin expression in esophageal cancer. *World J Gastroenterol.* 2006; 12:7104–7112. [PubMed: 17131471]
142. Du XL, et al. Proteomic profiling of proteins dysregulated in Chinese esophageal squamous cell carcinoma. *J Mol Med.* 2007; 85:863–875. [PubMed: 17318615]
143. Debelenko LV, et al. Renal cell carcinoma with novel *VCL-ALK* fusion: new representative of ALK-associated tumor spectrum. *Modern Pathol.* 2011; 24:430–442.
144. Sugawara E, et al. Identification of anaplastic lymphoma kinase fusions in renal cancer: large-scale immunohistochemical screening by the intercalated antibody-enhanced polymer method. *Cancer.* 2012; 118:4427–4436. [PubMed: 22252991]
145. Marino-Enriquez A, Ou WB, Weldon CB, Fletcher JA, Perez-Atayde AR. ALK rearrangement in sickle cell trait-associated renal medullary carcinoma. *Genes Chromosomes Cancer.* 2011; 50:146–153. [PubMed: 21213368]
146. Govindan R, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. *Cell.* 2012; 150:1121–1134. The authors undertook whole-genome and transcriptome sequencing of 17 NSCLC tumours and identified numerous genetic alterations, including 14 gene fusions. [PubMed: 22980976]
147. Suehara Y, et al. Identification of *KIF5B-RET* and *GOPC-ROS1* fusions in lung adenocarcinomas through a comprehensive mRNA-based screen for tyrosine kinase fusions. *Clin Cancer Res.* 2012; 18:6599–6608. [PubMed: 23052255]
148. Birch AH, et al. Chromosome 3 anomalies investigated by genome wide SNP analysis of benign, low malignant potential and low grade ovarian serous tumours. *PloS ONE.* 2011; 6:e28250. [PubMed: 22163003]
149. Lee J, et al. Identification of ROS1 rearrangement in gastric adenocarcinoma. *Cancer.* 2013
150. Grieco M, et al. PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected *in vivo* in human thyroid papillary carcinomas. *Cell.* 1990; 60:557–563. [PubMed: 2406025]
151. Bongarzone I, et al. Molecular characterization of a thyroid tumor-specific transforming sequence formed by the fusion of ret tyrosine kinase and the regulatory subunit RI alpha of cyclic AMP-dependent protein kinase A. *Mol Cell Biol.* 1993; 13:358–366. [PubMed: 7678053]
152. Bongarzone I, et al. Frequent activation of ret protooncogene by fusion with a new activating gene in papillary thyroid carcinomas. *Cancer Res.* 1994; 54:2979–2985. [PubMed: 8187085]
153. Klugbauer S, Demidchik EP, Lengfelder E, Rabes HM. Detection of a novel type of RET rearrangement (PTC5) in thyroid carcinomas after Chernobyl and analysis of the involved RET-fused gene RFG5. *Cancer Res.* 1998; 58:198–203. [PubMed: 9443391]

154. Nakata T, et al. Fusion of a novel gene, ELKS, to RET due to translocation t(10;12)(q11;p13) in a papillary thyroid carcinoma. *Genes Chromosomes Cancer*. 1999; 25:97–103. [PubMed: 10337992]
155. Corvi R, Berger N, Balczon R, Romeo G. RET/PCM-1: a novel fusion gene in papillary thyroid carcinoma. *Oncogene*. 2000; 19:4236–4242. [PubMed: 10980597]
156. Klugbauer S, Rabes HM. The transcription coactivator HTIF1 and a related protein are fused to the RET receptor tyrosine kinase in childhood papillary thyroid carcinomas. *Oncogene*. 1999; 18:4388–4393. [PubMed: 10439047]
157. Salassidis K, et al. Translocation t(10;14)(q11.2;q22.1) fusing the kinetin to the RET gene creates a novel rearranged form (PTC8) of the RET proto-oncogene in radiation-induced childhood papillary thyroid carcinoma. *Cancer Res*. 2000; 60:2786–2789. [PubMed: 10850414]
158. Saenko V, et al. Novel tumorigenic rearrangement, Delta rfp/ret, in a papillary thyroid carcinoma from externally irradiated patient. *Mut Res*. 2003; 527:81–90. [PubMed: 12787916]
159. Ciampi R, Giordano TJ, Wikenheiser-Brokamp K, Koenig RJ, Nikiforov YE. HOOK3-RET: a novel type of RET/PTC rearrangement in papillary thyroid carcinoma. *Endocr-Related Cancer*. 2007; 14:445–452.
160. Martin-Zanca D, Hughes SH, Barbacid M. A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. *Nature*. 1986; 319:743–748. [PubMed: 2869410]
161. Butti MG, et al. A sequence analysis of the genomic regions involved in the rearrangements between TPM3 and NTRK1 genes producing TRK oncogenes in papillary thyroid carcinomas. *Genomics*. 1995; 28:15–24. [PubMed: 7590742]
162. Greco A, et al. TRK-T1 is a novel oncogene formed by the fusion of TPR and TRK genes in human papillary thyroid carcinomas. *Oncogene*. 1992; 7:237–242. [PubMed: 1532241]
163. Greco A, et al. The DNA rearrangement that generates the TRK-T3 oncogene involves a novel gene on chromosome 3 whose product has a potential coiled-coil domain. *Mol Cell Biol*. 1995; 15:6118–6127. [PubMed: 7565764]
164. Skalova A, et al. Mammary analogue secretory carcinoma of salivary glands, containing the ETV6-NTRK3 fusion gene: a hitherto undescribed salivary gland tumor entity. *Am J Surg Pathol*. 2010; 34:599–608. [PubMed: 20410810]
165. Marsilje TH, et al. Synthesis, structure-activity relationships and *in vivo* efficacy of the novel potent and selective anaplastic lymphoma kinase (ALK) inhibitor 5-chloro-N2-(2-isopropoxy-5-methyl-4-(piperidin-4-yl)phenyl)-N4-(2-(isopropylsulfonyl)phenyl) pyrimidine-2,4-diamine (LDK378) currently in Phase 1 and 2 clinical trials. *J Med Chem*. 2013; 56:5675–5690. [PubMed: 23742252]
166. Patnaik A, et al. Pharmacokinetics and safety of an oral ALK inhibitor, ASP3026, observed in a phase I dose escalation trial. *J Clin Oncol Abstr*. 2013; 31:2602.
167. Lovly CM, et al. Insights into ALK-driven cancers revealed through development of novel ALK tyrosine kinase inhibitors. *Cancer Res*. 2011; 71:4920–4931. [PubMed: 21613408]
168. Wilcoxon KM, et al. Characterization of a novel series of potent, selective inhibitors of wild type and mutant/fusion anaplastic lymphoma kinase. *Cancer Res*. 2012; 72 (Suppl 1):1795. [PubMed: 22331459]
169. Gozgit JM, et al. Ponatinib is a highly potent inhibitor of activated variants of RET found in MTC and NSCLC. *Cancer Res*. 2013; 73 (Suppl 1):2084.
170. Plaza-Menacho I, et al. Sorafenib functions to potently suppress RET tyrosine kinase activity by direct enzymatic inhibition and promoting RET lysosomal degradation independent of proteasomal targeting. *J Biol Chem*. 2007; 282:29230–29240. [PubMed: 17664273]
171. Smyth EC, et al. FGFR: proof-of-concept study of AZD4547 in patients with FGFR1 or FGFR2 amplified tumors. *J Clin Oncol Abstr*. 2013; 31:TPS2626.
172. Gozgit JM, et al. Ponatinib (AP24534), a multitargeted pan-FGFR inhibitor with activity in multiple FGFR-amplified or mutated cancer models. *Mol Cancer Ther*. 2012; 11:690–699. [PubMed: 22238366]

173. Chase A, Grand FH, Cross NC. Activity of TKI258 against primary cells and cell lines with FGFR1 fusion genes associated with the 8p11 myeloproliferative syndrome. *Blood*. 2007; 110:3729–3734. [PubMed: 17698633]
174. Guagnano V, et al. FGFR genetic alterations predict for sensitivity to NVP-BGJ398, a selective pan-FGFR inhibitor. *Cancer Discov*. 2012; 2:1118–1133. [PubMed: 23002168]
175. Bello E, et al. E-3810 is a potent dual inhibitor of VEGFR and FGFR that exerts antitumor activity in multiple preclinical models. *Cancer Res*. 2011; 71:1396–1405. [PubMed: 21212416]
176. Yu Y, et al. Exploratory biomarker discovery for clinical development of ARQ 087, a potent pan-FGFR kinase inhibitor. *Cancer Res*. 2011; 71 (Suppl 1):3571.

Key points

- Chromosomal rearrangements that lead to oncogenic kinase activation are an emerging paradigm in epithelial cancers.
- Ten different tyrosine kinases have currently been identified as fusion kinases in various different epithelial tumours. Tyrosine kinase gene rearrangements generally define molecularly distinct subsets of patients.
- Cancers that harbour tyrosine kinase gene rearrangements express activated fusion kinases that drive the initiation and progression of malignancy. These cancers become dependent on (or 'addicted' to) continued signalling from the oncogenic fusion kinase.
- Several tyrosine kinase inhibitors have been shown to be active in cancers that harbour specific tyrosine kinase fusions, which validates these fusion kinases as bona fide targets. In the case of anaplastic lymphoma kinase (ALK)-rearranged lung cancers, ALK inhibitors like crizotinib have become a standard therapy.
- Multiplexed genetic analyses that include screening for tyrosine kinase fusions will help to accelerate the process of target discovery and clinical validation.

Figure 1. Molecular aetiology and types of chromosomal rearrangements

a | Several steps are thought to be required for the formation of a pathogenic fusion gene, symbolically shown here as A-B. First, double-strand breaks (DSBs) are initiated by cell-extrinsic mechanisms such as ionizing radiation or by various different cell-intrinsic mechanisms. Second, the ends of the broken DNA have to be brought into close proximity. This juxtaposition can occur after the formation of DSBs (as shown), or before the initiation of DSBs. Third, the DNA ends are aberrantly repaired, probably by alternative non-homologous end-joining (NHEJ). DNA junctions frequently show short stretches of homology that are referred to as microhomology and are indicated in the figure by the asterisk. In the final step, expression of the fusion gene confers a growth and/or survival advantage, which enables clonal selection and expansion. **b** | The boxes in the figure provide a summary of the different types of chromosomal rearrangements that lead to oncogenic tyrosine kinase fusions. Interchromosomal rearrangements, principally reciprocal translocations, are the most common type (left). However, intrachromosomal rearrangements (right), which include paracentric inversions, intrachromosomal deletion or tandem duplication, are also observed. All known anaplastic lymphoma kinase (ALK) and ROS1 fusions are shown here by the type of chromosomal rearrangement. Of note, complex rearrangements (not shown in the figure) may also lead to tyrosine kinase fusion genes. *C2orf44*, chromosome 2 open reading frame 44; *CCDC6*, coiled-coil domain-containing 6; CSR, class switch recombination; *EML4*, echinoderm microtubule-associated protein-like 4; *EZR*, villin 2; *FIG*, fused in glioblastoma; *KDEL2*, KDEL endoplasmic reticulum protein retention receptor 2; *KIF5B*, kinesin family member 5B; *KLC1*, kinesin light chain 1; *LRIG3*, leucine rich repeats and immunoglobulin-like domains 3; *SDC4*, syndecan 4;

SLC34A2, solute carrier family 34 member 2; *STRN*, striatin; *TFG*, TRK-fused gene; *TPM*, tropomyosin; *VCL*, vinculin; *VDJ*, variable diverse joining.

Figure 2. Genomic organization of tyrosine kinase rearrangements

Breakpoints (grey arrows), exons (drawn to scale) and introns are depicted. The numbering of the exons corresponds to coding, translated exons. Given the space limitations, sequences that correspond to untranslated regions are not depicted unless relevant (dashes). Regions that encode the transmembrane and kinase domains (as designated in UniProt) are shaded in blue and green, respectively, and were mapped using MapBack. The exon that encodes the GXGXXG motif is indicated with an asterisk. The isoform depicted for each kinase corresponds to the canonical isoform that is designated in UniProt unless a non-canonical isoform (for example, neurotrophic tyrosine kinase receptor type 3 (*NTRK3*)) or multiple isoforms (for example, fibroblast growth factor receptor 2 (*FGFR2*) and *FGFR3* (alternative exon 7, isoform number in superscript)) were specifically indicated in the primary reference. *ALK*, anaplastic lymphoma kinase; *PDGFRA*, platelet-derived growth factor receptor- α .

Table 1

Tyrosine kinase rearrangements in epithelial cancers

Kinase (location)	Malignancy	Rearrangement partners	Location of partners	Type of rearrangement	Frequency *	Refs
<i>ALK</i> (2p23)	NSCLC	<i>ML4</i>	2p21	Paracentric inversion	3–7%	3,4,69, 137–139
		<i>TFG</i>	3q12.2	Interchromosomal		
		<i>KIF5B</i>	10p11.22	Interchromosomal		
		<i>KLC1</i>	14q32.3	Interchromosomal		
		<i>STRN</i>	2p22.2	Deletion		
Colorectal cancer		<i>C2orf44</i>	2p23.3	Tandem duplication	<1%	56,130,140
		<i>ML4</i>	2p21	Paracentric inversion		
		<i>ML4</i>	2p21	Paracentric inversion	-	130
Breast cancer		<i>TPM4</i>	19p13.1	Interchromosomal	-	141,142
Oesophageal cancer (squamous cell)		<i>VCL</i>	10q22.2	Interchromosomal	<1%	143,144
Renal cell cancer		<i>TPM3</i>	1q21.2	Interchromosomal		
		<i>ML4</i>	2p21	Paracentric inversion		
		<i>VCL</i>	10q22.2	Interchromosomal	-	145
Renal medullary cancer	NSCLC	<i>SLC34A2</i>	4p15.2	Interchromosomal	1–2%	4,8, 68, 128,146, 147
		<i>CD74</i>	5q32	Interchromosomal		
		<i>TPM3</i>	1q21.2	Interchromosomal		
		<i>SDC4</i>	20q12	Interchromosomal		
		<i>EZR</i>	6q25.3	Paracentric inversion		
		<i>LRIG3</i>	12q14.1	Interchromosomal		
		<i>FIG</i>	6q21	Deletion		
		<i>KDEL2</i>	7p22.1	Interchromosomal		
		<i>CCDC6</i>	10q21	Interchromosomal		
		<i>FIG</i>	6q21	Deletion	8.7%	129
Cholangiocarcinoma		<i>FIG</i>	6q21	Deletion	-	148
Ovarian cancer		<i>SLC34A2</i>	4p15.2	Interchromosomal	-	149
Gastric cancer		<i>SLC34A2</i>	4p15.2	Interchromosomal	-	140

Kinase (location)	Malignancy	Rearrangement partners	Location of partners	Type of rearrangement	Frequency*	Refs
<i>RET</i> (10q11.2)	NSCLC	<i>KIF5B</i>	10p11.22	Pericentric inversion	1–2%	8,9,36, 57,110, 134,147
		<i>CCDC6</i>	10q21	Paracentric inversion		
		<i>NCOA4</i>	10q11.2	Paracentric inversion		
		<i>TRIM33</i>	1p13.1	Interchromosomal		
Papillary thyroid cancer		<i>CCDC6</i>	10q21	Paracentric inversion	~35% [‡]	150–159
		<i>PRKARIA</i>	17q24.2	Interchromosomal		
		<i>GOLGA5</i>	14q32.12	Interchromosomal		
		<i>NCOA4</i>	10q11.2	Paracentric inversion		
		<i>RAB6IP2</i>	12p13.3	Interchromosomal		
		<i>MBD1</i>	18q21	Interchromosomal		
		<i>TRIM24</i>	7q32-q34	Interchromosomal		
		<i>KTN1</i>	14q22.1	Interchromosomal		
		<i>TRIM33</i>	1p13.1	Interchromosomal		
		<i>TRIM27</i>	6p22	Interchromosomal		
		<i>HOOK3</i>	8p11.21	Interchromosomal		
<i>NTRK1</i> (1q21-22)	Colorectal cancer	<i>TPM3</i>	1q21.2	Paracentric inversion	-	160
Papillary thyroid cancer		<i>TPM3</i>	1q21.2	Paracentric inversion	12%	40,161, 162,163
		<i>TPR</i>	1q25	Paracentric inversion		
		<i>TFG</i>	3q12.2	Interchromosomal		
		<i>MPRIP</i>	17p11.2	Interchromosomal	-	135
Lung adenocarcinoma		<i>CD74</i>	5q32	Interchromosomal	-	
		<i>ETV6</i>	12p13	Interchromosomal	-	2
		<i>ETV6</i>	12p13	Interchromosomal	-	164
		<i>BAG4</i>	8p11.23	Intrachromosomal	-	64
<i>FGFR1</i> (8p12)	Squamous cell lung cancer	<i>ERLIN2</i>	8p11.2	Intrachromosomal	-	64
<i>FGFR2</i> (10q26)	Squamous cell lung cancer	<i>KIAA1967</i> [§]	8p22	Interchromosomal	-	64
		<i>CIT</i> [§]	12q24	Interchromosomal	-	68
		<i>AFF3</i> [§]	2q11.2-q12	Interchromosomal	-	64

Kinase (location)	Malignancy	Rearrangement partners	Location of partners	Type of rearrangement	Frequency*	Refs
		<i>CASP7</i> [§]	10q25	Intrachromosomal		
		<i>CCDC6</i> [§]	10q21	Intrachromosomal		
	Thyroid cancer	<i>OFD1</i> [§]	Xp22	Interchromosomal	-	64
	Prostate cancer	<i>SLC45A3</i>	1q32.1	Interchromosomal	-	64
	Cholangiocarcinoma	<i>BICC1</i> [§]	10q21.1	Intrachromosomal	-	64
		<i>TACC3</i> [§]	4p16.3	Intrachromosomal	-	64,114
	Bladder cancer	<i>BAIAP2L1</i> [§]	7q22.1	Interchromosomal		
		<i>TACC3</i> [§]	4p16.3	Intrachromosomal	-	64,69
<i>AXL</i> (19q13.1)	Lung adenocarcinoma	<i>MBIP</i> [§]	14q13.3	Interchromosomal	-	68
<i>PDGFRA</i> (4q12)	Lung adenocarcinoma	<i>SCAF11</i>	12q12	Interchromosomal	-	68

AFF3, AF4/FMR2 family, member 3; *ALK*, anaplastic lymphoma kinase; *BAG4*, BCL-2-associated anthanogene 4; *BAIAP2L1*, BAI1-associated protein 2-like 1; *BICC1*, bicaudal C homolog 1; *C2orf44*, chromosome 2 open reading frame 44; *CASP7*, caspase 7; *CCDC6*, coiled-coil domain-containing 6; *CIT*, citron (RHO-interacting, serine/threonine kinase 21); *EBL4*, echinoderm microtubule-associated protein-like 4; *ERLIN2*, endoplasmic reticulum lipid raft associated 2; *ETV6*, ETS variant 6; *EZR*, villin 2; *FGFR*, fibroblast growth factor receptor; *GOLGA5*, golgin A5; *FIG*, fused in glioblastoma; *KDELR2*, KDEL endoplasmic reticulum protein retention receptor 2; *KIF5B*, kinesin family member 5B; *KLC1*, kinesin light chain 1; *KTN1*, kinesin; *LRIG3*, leucine-rich repeats and immunoglobulin-like domains 3; *MBD1*, methyl CpG binding domain protein 1; *MBIP*, MAP3K12-binding inhibitory protein; *MPRI*, gene encoding myosin phosphatase RHO-interacting protein; *NCOA4*, nuclear receptor coactivator 4; *NTRK3*, neurotrophic tyrosine kinase receptor type 3; *NSCLC*, non-small-cell lung cancer; *OFD1*, oral-facial-digital syndrome 1; *PDGFRA*, platelet-derived growth factor receptor-a; *PRKARIA*, cAMP-dependent protein kinase type 1a; *RAB6IP2*, RAB6, interacting protein 2; *SCAF11*, SR-related C-terminal domain-associated factor 11; *SDC4*, syndecan 4; *SLC*, solute carrier protein; *STRN*, striatin; *TACC3*, transforming acidic coiled-coil-containing protein 3; *TFG*, TRK-fused gene; *TPM*, tropomyosin; *TRIM*, tripartite motif containing protein; *VCL*, vinculin.

* Estimated frequency is shown if frequency of the fusion kinase was reported in two or more studies.

[‡] Ranges from 3% to 85% across studies.

[§] For these fusions, the 5 fusion partner is the tyrosine kinase gene.

Table 2

ALK and/or ROS1 tyrosine kinase inhibitors in the clinic

Drug	Company	ROS1 activity?	Status	Ongoing studies	NCT identifier*	Refs
Crizotinib	Pfizer	Yes	Approved for ALK-positive NSCLC; investigational for ROS1	Phase I for ROS1 and MET Phase III for ALK-positive NSCLC comparing crizotinib with first-line chemotherapy	00585195 01154140	86, 105
LDK378	Novartis	Yes	Investigational (breakthrough therapy designation)	Phase I for ALK Phase II for ALK-positive NSCLC, crizotinib-naïve Phase II for ALK-positive NSCLC, crizotinib-treated Phase III for ALK-positive NSCLC comparing LDK378 with chemotherapy, crizotinib-naïve Phase III for ALK-positive NSCLC comparing LDK378 with chemotherapy, crizotinib-treated	01283516 01685138 01685060 01828099 01828112	100, 165
CH5424802	Chugai	No	Investigational	Phase I and Phase II study for ALK	01588028	97
AP26113	Ariad	Yes	Investigational	Phase I and Phase II for ALK, ROS1 and other solid tumours	01449461	99
ASP3026	Astellas	Yes	Investigational	Phase I for ALK, ROS1 and other solid tumours	01284192	166
X-396	Xcovery	Yes	Investigational	Phase I for ALK and other solid tumours	01625234	167
TSR-011	Tesaro	Unknown	Investigational	Phase I and Phase II for ALK		168

ALK, anaplastic lymphoma kinase; NSCLC, non-small-cell lung cancer.

* See the ClinicalTrials.gov website.

Table 3

RET tyrosine kinase inhibitors in the clinic

Drug	Company	Other targets	Status	Ongoing studies	NCT identifier*	Refs
Cabozantinib	Exelixis	• VEGFR2	Approved for medullary thyroid cancer (MTC)	Phase II for RET-rearranged NSCLC	01639508	110
		• MET		Phase II for refractory differentiated thyroid cancer	01811212	
Vandetanib	AstraZeneca	• VEGFR	Approved for MTC	Phase II for RET-rearranged NSCLC	01823068	111
		• EGFR				
Ponatinib	Ariad	• BCR-ABL	Approved for resistant CML and Ph ⁺ ALL	Phase II for RET-rearranged NSCLC	01813734	169
		• FGFR1		Phase II for selected NSCLC, including RET-rearranged NSCLC	01935336	
		• PDGFR				
		• FLT3				
		• VEGFR2				
Sunitinib	Pfizer	• KIT	Approved for renal cell carcinoma and imatinib-resistant GIST	Phase II for never-smokers with lung adenocarcinoma, including RET-rearranged NSCLC	01829217	56
		• VEGFR		Phase II for refractory differentiated thyroid cancer and MTC	00381641	
		• PDGFR				
		• KIT				
		• FLT3				
Sorafenib	Bayer/Onyx	• VEGFR	Approved for advanced renal cell carcinoma and hepatocellular carcinoma	Phase II for younger patients with select cancers, including recurrent thyroid cancer	01502410	170
		• BRAF				
		• CRAF				
		• KIT				

ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; CSF1, colony-stimulating factor receptor 1; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; FLT3, FMS-like tyrosine kinase 3; GIST, gastrointestinal stromal tumour; NSCLC, non-small-cell lung cancer; PDGFR, platelet-derived growth factor receptor; Ph⁺, Philadelphia chromosome positive; VEGFR, vascular endothelial growth factor receptor.

* See the ClinicalTrials.gov website.

Table 4

FGFR tyrosine kinase inhibitors in the clinic

Drug	Company	Targets	Status	Ongoing studies	NCT identifier*	Refs
AZD4547	AstraZeneca	FGFR1–FGFR3	Investigational	Phase II for FGFR1- or FGFR2-amplified cancers	01795768	171
Ponatinib	Ariad	<ul style="list-style-type: none"> • BCR–ABL • FGFR1–FGFR4 • RET • PDGFR • FLT3 • VEGFR2 • KIT 	Approved for resistant CML and Ph ⁺ ALL	Phase II for advanced squamous cell carcinoma Phase II for selected NSCLC	01761747 01935336	172
Dovitinib	Novartis	<ul style="list-style-type: none"> • FGFR1–FGFR3 • PDGFR • VEGFR • FLT3 • KIT 	Investigational	Phase II for cancers with mutations or translocations of FGFR or other kinases Phase II for FGFR1-amplified squamous cell lung cancer Phase II for advanced urothelial cancers with FGFR3 mutations or overexpression Phase II for advanced gastric cancers with FGFR2 amplification Phase II for metastatic endometrial cancers	01831726 01861197 01732107 01719549 01379534	173
BGJ398	Novartis	FGFR1–FGFR3	Investigational	Phase I for advanced cancers with FGFR1 or FGFR2 amplification, or with FGFR3 mutation	01004224	174
E-3810	EOS	<ul style="list-style-type: none"> • FGFR1–FGFR2 • VEGFR 	Investigational	Phase I for advanced solid tumours	01283945	175
INJ-42756493	Astex/Janssen		Investigational	Phase I for advanced solid tumours and lymphoma, with FGFR1, FGFR2 or FGFR4 amplification required in expansion phase	01703481	
ARQ 087	ArQule		Investigational	Phase I for advanced solid tumours	01752920	176

ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; FGFR, fibroblast growth factor receptor; FLT3, FMS-like tyrosine kinase 3; PDGFR, platelet-derived growth factor receptor; Ph⁺, Philadelphia chromosome positive; VEGFR, vascular endothelial growth factor receptor.

* See the ClinicalTrials.gov website.