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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 cancer[1]. 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 cancer[2]. 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 survival[5]. 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

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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 cancers[124]. 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 development[126]. 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 karyotypes[6]. 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 States[7], but they are found in only 1% of NSCLCs[8,9]. *ROS1* fusions are similarly rare in NSCLC[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 year[12], 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 radiation[26].

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 increased[34]. It has also been suggested that oxidative stress contributes to chromosomal instability[35]. 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 cancer[7], which include both radiation- and non-radiation-associated cases[40]. In the case of radiation-induced *RET* rearrangements, the increased susceptibility of children compared to adults has been attributed to more rapid thyroid cell proliferation[41].

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 reviewed[51], 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 cells[52]. 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 cells[52], 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 amplicons[55]. 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 β1 (*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 activity[59] (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 exon[59] (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-a (*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 domains[8]. 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 compartments[7]. 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 aneuploidy[72]. 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 clinic[86]. 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 (IC_{50}) of 8 nM and 20 nM, respectively[89]. 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 study[92]. Results from the first Phase III randomized study of crizotinib were recently reported[94]. 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 NSCLC[3]. 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 ALK[95]. In cell-free enzymatic assays, CH5424802 was highly potent against ALK; it had an IC_{50} 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 crizotinibnaive, *ALK*-rearranged NSCLC[97]. 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 population[98]. 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.

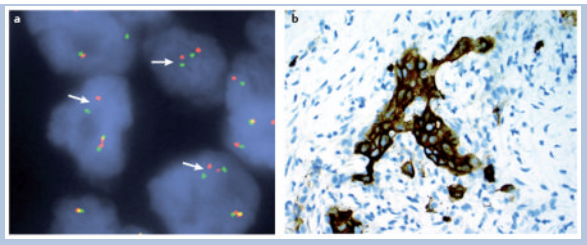
Box 2

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 States[86]; 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* FISH[128]. 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 PFS[100]. 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 extents[103]. 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 kinases[104]. 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 crizotinib[103]. 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 NSCLC[96], 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 NSCLC[110]. 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 NSCLC[111]. 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 pazopanib[64]. 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 fusion[72].

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 proteins[115]. 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 signalling[116]. 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 NSCLC[117]. 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 inhibitor[119].

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 protein[121]. 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 apoptosis[120]. Ganetespib is also active in various models of *ROS1*-rearranged cancers, including the lung cancer cell line HCC78, which harbours the *SLC34A2–ROS1* fusion[120]. 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 crizotinibnaive, and each patient received IPI-504 for approximately 7 months[117]. High response rates have also been reported with ganetespib and AUY922 in crizotinib-naive, *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-naive 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 ganetespib[120], and in 3 out of 11 patients (27%) who were treated with AUY922 (REF. 123). Second, in either treatment-naive 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-naive 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 oncogene[1]. 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 patient[3]. 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) samples[4]. Novel candidate driver kinases were identified using this analysis, including ALK, ROS1, platelet-derived growth factor receptor-a (PDGFRa) 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 cholangiocarcinoma[129].

Using breakpoint analysis of exon array data, *EML4–ALK* fusion transcripts were discovered not only in NSCLC but also in breast and colorectal cancer samples[130]. 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 drivers[131]. 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 assay[132]. Existing exon array and comparative genomic hybridization (CGH) data sets have most recently been mined to identify rearrangements, such as *CEP85L–ROS1* in angiosarcoma[133].

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 tumours[64], 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 adenocarcinoma[69]. 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 samples[56]. 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 method[135].

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 motifs[59]. In a leukaemia patient with no known gene rearrangements, this technique was used to identify a novel *CEP85L–PDGFRB* fusion[136]. 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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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.

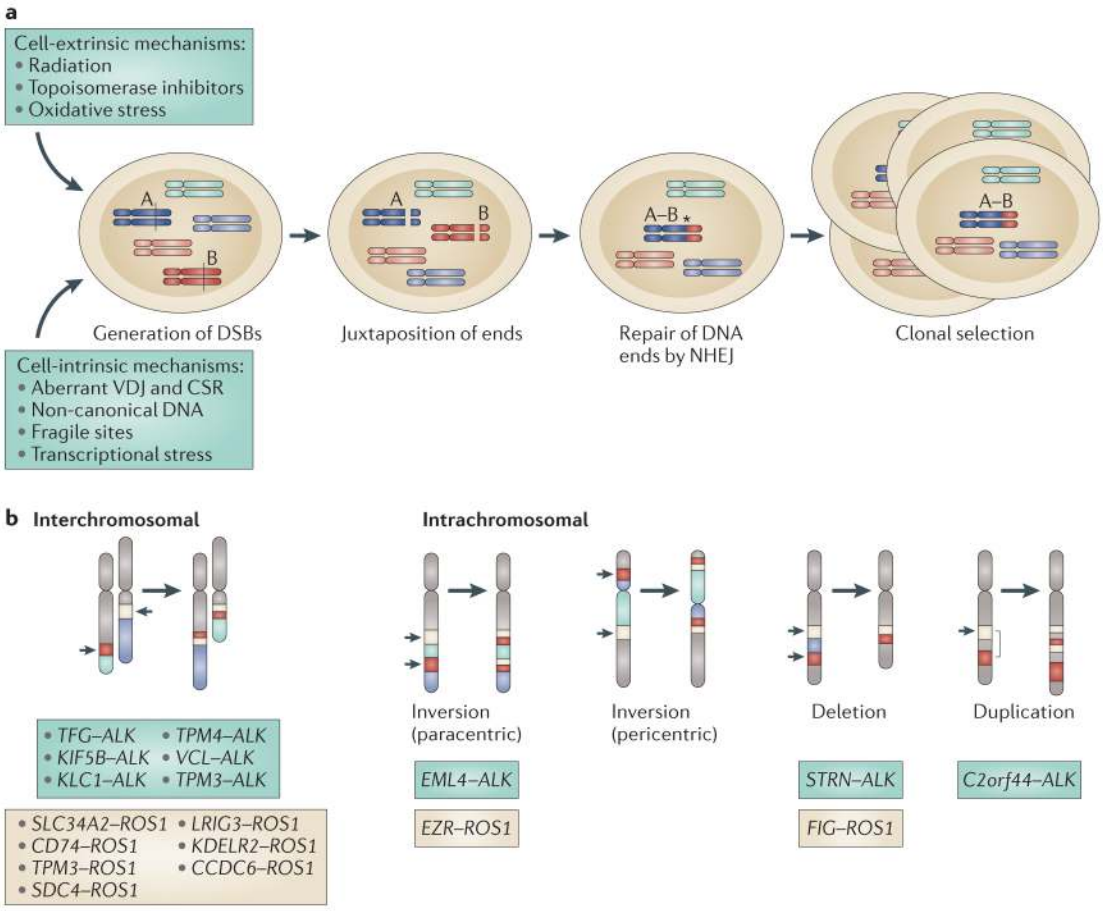

Figure 1. Molecular aetiology and types of chromosomal rearrangements

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; *KDELR2*, 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*, tropomysin; VCL, vinculin; VDJ, variable diverse joining.

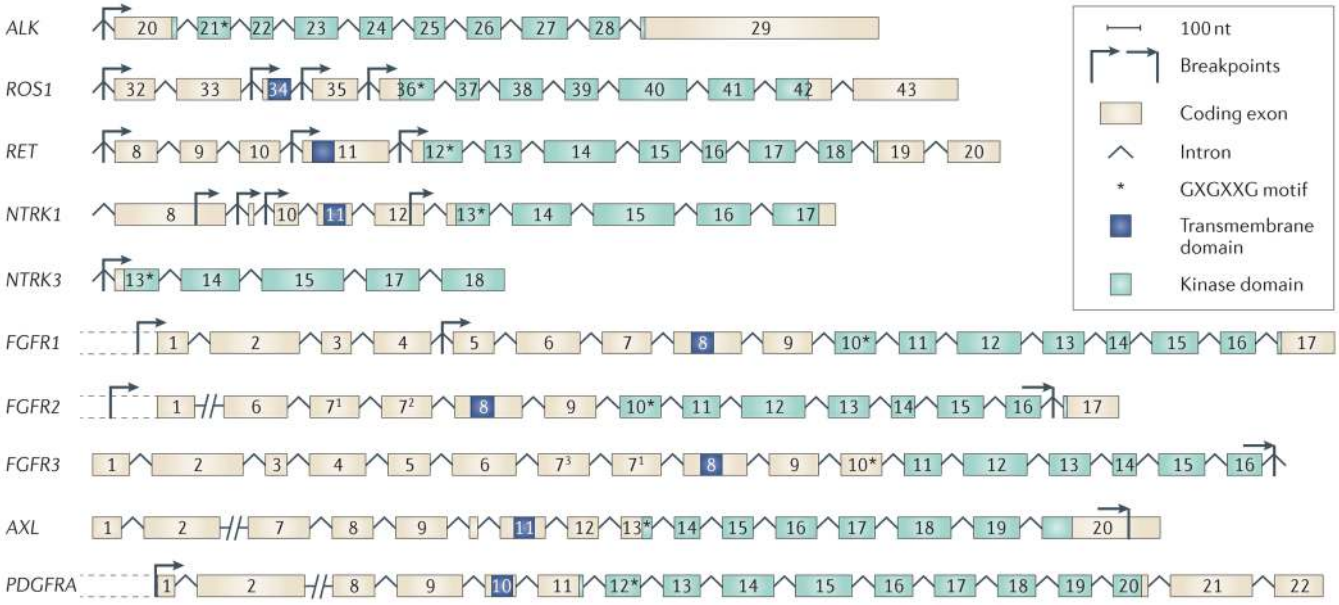

Figure 2. Genomic organization of tyrosine kinase rearrangements

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-a.

Table 1

Tyrosine kinase rearrangements in epithelial cancers

Kinase (location)	Malignancy	Rearrangement partners	Location of partners	Type of rearrangement	Frequency*	Refs
ALK (2p23)	NSCLC	*EML4*	2p21	Paracentric inversion	3–7%	3,4,69, 137–139
		TFG	3q12.2	Interchromosomal		
		KIF5B	10p11.22	Interchromosomal		
		KLC1	14q32.3	Interchromosomal		
		STRN	2p22.2	Deletion		
	Colorectal cancer	*C2orf44*	2p23.3	Tandem duplication	<1%	56,130,140
		EML4	2p21	Paracentric inversion		
	Breast cancer	*EML4*	2p21	Paracentric inversion	-	130
	Oesophageal cancer (squamous cell)	*TPM4*	19p13.1	Interchromosomal	-	141,142
	Renal cell cancer	*VCL*	10q22.2	Interchromosomal	<1%	143,144
		TPM3	1q21.2	Interchromosomal		
		EML4	2p21	Paracentric inversion		
	Renal medullary cancer	*VCL*	10q22.2	Interchromosomal	-	145
ROS1 (6q22)	NSCLC	*SLC34A2*	4p15.2	Interchromosomal	1–2%	4,8, 68, 128,146, 147
		CD74	5q32	Interchromosomal		
		TPM3	1q21.2	Interchromosomal		
		SDC4	20q12	Interchromosomal		
		EZR	6q25.3	Paracentric inversion		
		LRIG3	12q14.1	Interchromosomal		
		FIG	6q21	Deletion		
		KDELR2	7p22.1	Interchromosomal		
		CCDC6	10q21	Interchromosomal		
	Cholangiocarcinoma	*FIG*	6q21	Deletion	8.7%	129
	Ovarian cancer	*FIG*	6q21	Deletion	-	148
	Gastric cancer	*SLC34A2*	4p15.2	Interchromosomal	-	149
	Colorectal cancer	*SLC34A2*	4p15.2	Interchromosomal	-	140

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Kinase (location)	Malignancy	Rearrangement partners	Location of partners	Type of rearrangement	Frequency*	Refs
RET (10q11.2)	NSCLC	*KIF5B*	10p11.22	Pericentric inversion	1–2%	8,9,56, 57,110, 134,147
		CCDC6	10q21	Paracentric inversion		
		NCOA4	10q11.2	Paracentric inversion		
		TRIM33	1p13.1	Interchromosomal		
	Papillary thyroid cancer	*CCDC6*	10q21	Paracentric inversion	~35%‡	150–159
		PRKAR1A	17q24.2	Interchromosomal		
		GOLGA5	14q32.12	Interchromosomal		
		NCOA4	10q11.2	Paracentric inversion		
		RAB6IP2	12p13.3	Interchromosomal		
		MBD1	18q21	Interchromosomal		
		TRIM24	7q32-q34	Interchromosomal		
		KTN1	14q22.1	Interchromosomal		
		TRIM33	1p13.1	Interchromosomal		
		TRIM27	6p22	Interchromosomal		
		HOOK3	8p11.21	Interchromosomal		
NTRK1 (1q21-22)	Colorectal cancer	*TPM3*	1q21.2	Paracentric inversion	-	160
	Papillary thyroid cancer	*TPM3*	1q21.2	Paracentric inversion	12%	40,161, 162,163
		TPR	1q25	Paracentric inversion		
		TFG	3q12.2	Interchromosomal		
	Lung adenocarcinoma	*MPRIP*	17p11.2	Interchromosomal	-	135
		CD74	5q32	Interchromosomal	-	
NTRK3 (15q25)	Secretory breast cancer	*ETV6*	12p13	Interchromosomal	-	2
	Salivary gland tumour	*ETV6*	12p13	Interchromosomal	-	164
FGFR1 (8p12)	Squamous cell lung cancer	*BAG4*	8p11.23	Intrachromosomal	-	64
	Breast cancer	*ERLIN2*	8p11.2	Intrachromosomal	-	64
FGFR2 (10q26)	Squamous cell lung cancer	*KIAA1967*§	8p22	Interchromosomal	-	64
	Lung adenocarcinoma	*CIT*§	12q24	Interchromosomal	-	68
	Breast cancer	*AFF3*§	2q11.2-q12	Interchromosomal	-	64

Kinase (location)	Malignancy	Rearrangement partners	Location of partners	Type of rearrangement	Frequency*	Refs
		CASP7§	10q25	Intrachromosomal		
		CCDC6§	10q21	Intrachromosomal		
	Thyroid cancer	*OFD1*§	Xp22	Interchromosomal	-	64
	Prostate cancer	*SLC45A3*	1q32.1	Interchromosomal	-	64
	Cholangiocarcinoma	*BICC1*§	10q21.1	Intrachromosomal	-	64
FGFR3 (4p16.3)	Bladder cancer	*TACC3*§	4p16.3	Intrachromosomal	-	64,114
		BAIAP2L1§	7q22.1	Interchromosomal		
	Squamous cell carcinoma (lung, head and neck)	*TACC3*§	4p16.3	Intrachromosomal	-	64,69
AXL (19q13.1)	Lung adenocarcinoma	*MBIP*§	14q13.3	Interchromosomal	-	68
PDGFRA (4q12)	Lung adenocarcinoma	*SCAF11*	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); *EML4*, 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*, kinectin; *LRIG3*, leucine-rich repeats and immunoglobulin-like domains 3; *MBD1*, methyl CpG binding domain protein 1; *MBIP*, MAP3K12-binding inhibitory protein; *MPRIP*, 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; *PRKAR1A*, 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	00585195	86, 105
				Phase III for ALK-positive NSCLC comparing crizotinib with first-line chemotherapy	01154140	
LDK378	Novartis	Yes	Investigational (breakthrough therapy designation)	Phase I for ALK	01283516	100, 165
				Phase II for ALK-positive NSCLC, crizotinib-naive	01685138	
				Phase II for ALK-positive NSCLC, crizotinib-treated	01685060	
				Phase III for ALK-positive NSCLC comparing LDK378 with chemotherapy, crizotinib-naive	01828099	
				Phase III for ALK-positive NSCLC comparing LDK378 with chemotherapy, crizotinib-treated	01828112	
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 • MET	Approved for medullary thyroid cancer (MTC)	Phase II for RET-rearranged NSCLC	01639508	110
				Phase II for refractory differentiated thyroid cancer	01811212	
Vandetanib	AstraZeneca	• VEGFR • EGFR	Approved for MTC	Phase II for RET-rearranged NSCLC	01823068	111
Ponatinib	Ariad	• BCR–ABL • FGFR1 • PDGFR • FLT3 • VEGFR2 • KIT	Approved for resistant CML and Ph^+ ALL	Phase II for RET-rearranged NSCLC	01813734	169
				Phase II for selected NSCLC, including RET-rearranged NSCLC	01935336	
Sunitinib	Pfizer	• VEGFR • PDGFR • KIT • FLT3 • CSF1R	Approved for renal cell carcinoma and imatinib-resistant GIST	Phase II for never-smokers with lung adenocarcinoma, including RET-rearranged NSCLC	01829217	56
				Phase II for refractory differentiated thyroid cancer and MTC	00381641	
Sorafenib	Bayer/Onyx	• VEGFR • BRAF • CRAF • KIT	Approved for advanced renal cell carcinoma and hepatocellular carcinoma	Phase II for younger patients with select cancers, including recurrent thyroid cancer	01502410	170

ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; CSFR1, 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	• BCR–ABL • FGFR1– FGFR4 • RET • PDGFR • FLT3 • VEGFR2 • KIT	Approved for resistant CML and Ph+ ALL	Phase II for advanced squamous cell carcinoma	01761747	172
				Phase II for selected NSCLC	01935336	
Dovitinib	Novartis	• FGFR1– FGFR3 • PDGFR • VEGFR • FLT3 • KIT	Investigational	Phase II for cancers with mutations or translocations of FGFR or other kinases	01831726	173
				Phase II for FGFR1-amplified squamous cell lung cancer	01861197	
				Phase II for advanced urothelial cancers with FGFR3 mutations or overexpression	01732107	
				Phase II for advanced gastric cancers with FGFR2 amplification	01719549	
				Phase II for metastatic endometrial cancers	01379534	
BGJ398	Novartis	FGFR1– FGFR3	Investigational	Phase I for advanced cancers with FGFR1 or FGFR2 amplification, or with FGFR3 mutation	01004224	174
E-3810	EOS	• FGFR1– FGFR2 • VEGFR	Investigational	Phase I for advanced solid tumours	01283945	175
JNJ-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.