NOVEL KIF5B-RET FUSION MOLECULES AND USES THEREOF

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/526,613, filed August 23, 2011; U.S. Provisional Application No. 61/537,024, filed September 20, 2011; U.S. Provisional Application No. 61/542,112, filed September 30, 2011; and U.S. Provisional Application No. 61/594,739, filed February 3, 2012. The contents of all these prior applications are incorporated herein by reference in their entirety.

BACKGROUND

Cancer represents the phenotypic end-point of multiple genetic lesions that endow cells with a full range of biological properties required for tumorigenesis. Indeed, a hallmark genomic feature of many cancers, including, for example, B cell cancer, lung cancer, breast cancer, ovarian cancer, pancreatic cancer, and colon cancer, is the presence of numerous complex chromosome structural aberrations, including translocations, intra-chromosomal inversions, point mutations, deletions, gene copy number changes, gene expression level changes, and germline mutations, among others.

The need still exists for identifying novel genetic lesions associated with cancer. Such genetic lesions can be an effective approach to develop compositions, methods and assays for evaluating and treating cancer patients.

SUMMARY

The disclosure is based, at least in part, on the discovery of novel inversion events that include a fragment of a KIF5B gene ("Kinesin Family Member 5B- RET proto-oncogene") and a fragment of a RET proto-oncogene in a cancer, *e.g.*, a lung cancer, referred to herein as "KIF5B-RET." The term "KIF5B-RET" or "KIF5B-RET fusion" is used generically herein, and includes any fusion molecule (*e.g.*, gene, gene product (*e.g.*, cDNA, mRNA, or polypeptide), and variant thereof) that includes a fragment of KIF5B and a fragment of RET, including, *e.g.*, a 5'KIF5B-3'RET.

In one example, a KIF5B-RET fusion includes an in-frame fusion of an exon of KIF5B (*e.g.*, one more exons encoding a kinesin motor domain or a fragment thereof)

and an exon of RET (*e.g.*, one or more exons encoding a RET tyrosine kinase domain or a fragment thereof). For example, the KIF5B-RET fusion can include an in-frame fusion of at least exon 15 of KIF5B or a fragment thereof (*e.g.*, exons 1-15 of KIF5B or a fragment thereof) with at least exon 12 of RET or a fragment thereof (*e.g.*, exons 12-20 of RET or a fragment thereof). In certain examples, the KIF5B-RET fusion is in a 5'-KIF5B to 3'-RET configuration referred to herein as "5'KIF5B-3'RET." A KIF5B-RET fusion polypeptide encoded by a 5'KIF5B-3'RET nucleic acid is sometimes referred to herein as a 5'KIF5B-3'RET polypeptide. In an example, the 5'KIF5B-3'RET fusion comprises sufficient KIF5B and sufficient RET sequence such that the 5'KIF5B-3'RET fusion has kinase activity, e.g., has elevated activity, *e.g.*, kinase activity, as compared with wild type RET, *e.g.*, in a cell of a cancer referred to herein. In one example, the 5'KIF5B-3'RET fusion comprises at least 1, 2, 3, 4, 5, 6, 7, 9, 10, or 11 exons from KIF5B and at least 1, 2, 3, 4, 5, 6, 7, 9, or 10, RET exons. In one example, the 5'KIF5B-3'RET fusion polypeptide includes a kinesin motor domain, a coiled coil domain, or a functional fragment thereof, and a RET tyrosine kinase domain or a functional fragment thereof.

The RET proto-oncogene is associated with cancerous phenotypes, including papillary thyroid carcinomas (PTC), multiple endocrine neoplasias (MEN), phaeochromocytoma, among others. For example, chromosomal rearrangements that generate a fusion gene resulting in the juxtaposition of the C-terminal region of the RET protein with an N-terminal portion of another protein (known as RET/PTC) are known to be associated with PTC (Nikiforov, YE (2002) *Endocr. Pathol.* 13 (1):3-16). The RET/PTC has been shown to cause constitute activation of the RET kinase domain, which is a likely contributor to tumorigenicity. The KIF5B-RET fusions disclosed herein (*e.g.*, the 5'- KIF5B to 3'-RET fusions that include a RET tyrosine kinase domain) are associated with cancers, *e.g.*, lung cancer. Elevated expression of the 3' end of RET beginning in exon 12 is detected in lung tumor samples, suggesting that the KIF5B-RET fusion transcript can result in RET kinase domain overexpression.

In other examples, the KIF5B-RET fusion includes an in-frame fusion of at least exon 11 of RET or a fragment thereof (*e.g.*, exons 1-11 of RET or a fragment thereof) with at least exon 16 or a fragment thereof (*e.g.*, exons 16-25 of KIF5B or a fragment thereof). In certain examples, the KIF5B-RET fusion is in a 5'- RET to 3'-

KIF5B configuration referred to herein as "5'RET-3'KIF5B"). The 5'RET-3'KIF5B configuration of the fusion molecule is not believed to be expressed.

Accordingly, the disclosure provides, methods of: identifying, assessing or detecting a KIF5B-RET fusion; methods of identifying, assessing, evaluating, and/or treating a subject having a cancer, *e.g.*, a cancer having a KIF5B-RET fusion; isolated KIF5B-RET nucleic acid molecules, nucleic acid constructs, host cells containing the nucleic acid molecules; purified KIF5B-RET polypeptides and binding agents; detection reagents (*e.g.*, probes, primers, antibodies, kits, capable, e.g., of specific detection of a KIF5B-RET nucleic acid or protein); screening assays for identifying molecules that interact with, e.g., inhibit, 5'KIF5B-3'RET fusions, e.g., novel kinase inhibitors; as well as assays and kits for evaluating, identifying, assessing and/or treating a subject having a cancer, *e.g.*, a cancer having a KIF5B-RET fusion. The compositions and methods identified herein can be used, for example, to identify new KIF5B-RET inhibitors; to evaluate, identify or select subject, *e.g.*, a patient, having a cancer; and to treat or prevent a cancer.

KIF5B-RET Nucleic Acid Molecules

In one example, the disclosure features a nucleic acid molecule (*e.g.*, an isolated or purified) nucleic acid molecule that includes a fragment of a KIF5B gene and a fragment of a RET proto-oncogene. In one example, the nucleic acid molecule includes a fusion, *e.g.*, an in-frame fusion, of an exon of KIF5B (*e.g.*, one more exons encoding a kinesin motor domain or a fragment thereof), and an exon of RET (*e.g.*, one or more exons encoding a RET tyrosine kinase domain or a fragment thereof).

In an example the 5'KIF5B-3'RET nucleic acid molecule comprises sufficient KIF5B and sufficient RET sequence such that the encoded 5'KIF5B-3'RET fusion has kinase activity, *e.g.*, has elevated activity, *e.g.*, kinase activity, as compared with wild type RET, e.g., in a cell of a cancer referred to herein. In an example the encoded 5'KIF5B-3'RET fusion comprises at least 1, 2, 3, 4, 5, 6, 7, 9, 10, or 11 exons from KIF5B and at least 1, 2, 3, 4, 5, 6, 7, 9, or 10, RET exons. In one example, the encoded 5'KIF5B-3'RET fusion polypeptide includes a kinesin motor domain, a coiled coil domain, or a functional fragment thereof, and a RET tyrosine kinase domain or a functional fragment thereof.

In one example, the nucleic acid molecule includes a nucleotide sequence that has an in-frame fusion of exon 15 of KIF5B with exon 12 of RET (*e.g.*, a sequence within an 11MB pericentric inversion on chromosome 10). In other examples, the nucleic acid molecules includes a nucleotide sequence in the region of 32,316,376-32,316,416 of chromosome 10 coupled to (*e.g.,* juxtaposed to) nucleotides in the region of nucleotides 43,611,042-43,611,118 of chromosome 10. In another example, the nucleic acid molecule includes a nucleotide sequence that includes a breakpoint, *e.g.*, a breakpoint identified in FIGs. 1, 2 and 3A-3D. For example, the nucleic acid molecule includes a nucleotide sequence that includes the fusion junction between the KIF5B transcript and the RET transcript, *e.g.*, a nucleotide sequence that includes a portion of SEQ ID NO:1 (*e.g.*, a nucleotide sequence within exons 1-15 of a KIF5B gene and 12-20 of a RET gene) (*e.g.*, a portion of SEQ ID NO:1 comprising nucleotides 1720-1731, 1717-1734, or 1714-1737 of SEQ ID NO:1 (see FIG. 3B)).

In other examples, the nucleic acid molecule includes a KIF5B-RET fusion having a configuration shown in FIGs. 2B and 3A-3D. For example, the KIF5B-RET fusion can include an in-frame fusion of at least exon 15 of KIF5B or a fragment thereof (*e.g.*, exons 1-15 of KIF5B or a fragment thereof) with at least exon 12 of RET or a fragment thereof (*e.g.*, exons 12-20 of RET or a fragment thereof). In certain examples, the KIF5B-RET fusion is in a 5'-KIF5B to 3'-RET configuration referred to herein as "5'KIF5B-3'RET"). In one example, the nucleic acid molecule includes the nucleotide sequence of nucleotides 1-1725 of SEQ ID NO:1 (corresponding to exons 1-15 of the KIF5B gene), or a fragment thereof, or a sequence substantially identical thereto. In another example, the nucleic acid molecule includes the nucleotide sequence of nucleotides 1726-2934 of SEQ ID NO:1 (*e.g.*, corresponding to exons 12-20 of the RET gene), or a fragment thereof, or a sequence substantially identical thereto. In yet other examples, the nucleic acid molecule includes the nucleotide sequence shown in FIGs. 3A-3D (*e.g.*, SEQ ID NO:1) or a fragment thereof, or a sequence substantially identical thereto. In one example, the nucleic acid molecule is complementary to at least a portion of a nucleotide sequence disclosed herein, *e.g.*, is capable of hybridizing under a stringency condition described herein to SEQ ID NO:1 or a fragment thereof. In yet other example, the nucleic acid molecule hybridizes to a nucleotide sequence that is complementary to at least a portion of a nucleotide sequence

disclosed herein, *e.g.*, is capable of hybridizing under a stringency condition described herein to a nucleotide sequence complementary to SEQ ID NO:1 or a fragment thereof. The nucleotide sequence of a cDNA encoding an exemplary 5'KIF5B-3'RET fusion is shown in SEQ ID NO:1, and the amino acid sequence is shown in SEQ ID NO:2.

In other examples, the nucleic acid molecule includes a nucleotide sequence encoding a KIF5B-RET fusion polypeptide that includes a fragment of a KIF5B gene and a fragment of a RET proto-oncogene. In one example, the nucleotide sequence encodes a KIF5B-RET fusion polypeptide that includes a kinesin motor domain or a functional fragment thereof, and a RET tyrosine kinase domain or a functional fragment thereof. In another example, the nucleotide sequence encodes a fragment of the KIF5B polypeptide including the amino acid sequence of amino acids 1-575 of SEQ ID NO:2 or a fragment thereof, or a sequence substantially identical thereto. For example, the nucleic acid molecule can include a nucleotide sequence encoding a kinesin motor domain of KIF5B-RET fusion polypeptide that includes amino acids 6-325 of SEQ ID NO:2 or a fragment thereof. In other examples, the nucleic acid molecule includes a fragment of the RET gene encoding the amino acid sequence of amino acids 576-977 of SEQ ID NO:2 or a fragment thereof, or a sequence substantially identical thereto. For example, the nucleic acid molecule can include a nucleotide sequence encoding a RET kinase domain of a KIF5B-RET fusion polypeptide that includes amino acids 587-868 of SEQ ID NO:2 or a fragment thereof. In yet other examples, the nucleic acid molecule includes a nucleotide sequence encoding the amino acid sequence shown in FIGs. 3A-3D (*e.g.*, SEQ ID NO:2) or a fragment thereof, or a sequence substantially identical thereto.

In another example, the nucleic acid molecule includes a KIF5B-RET fusion having the configuration shown in FIGs. 2A and 4A-4D. In one example, the nucleic acid molecule includes a nucleotide sequence that include a fusion junction between the RET transcript and the KIF5B transcript, *e.g.*, a nucleotide sequence within SEQ ID NO:3 (*e.g.*, a sequence comprising nucleotides 2131-2142, 2128-2145, or 2125-2148 of SEQ ID NO:3 (see FIG. 4B)). In another example, the nucleic acid molecule includes a fusion, *e.g.*, an in-frame fusion, of at least exon 11 of RET or a fragment thereof (*e.g.*, exons 1-11 of RET or a fragment thereof), and at least exon 16 or a fragment thereof (*e.g.*, exons 16-25 of KIF5B or a fragment thereof). In certain

examples, the KIF5B-RET fusion is in a 5'- RET to 3'- KIF5B configuration referred to herein as "5'RET-3'KIF5B"). In one example, the nucleic acid molecule includes the nucleotides sequence of 1-2136 of SEQ ID NO:3 (corresponding to exons 1-11 of a RET gene) or a fragment thereof, or a sequence substantially identical thereto. In another example, the nucleic acid molecule includes the nucleotides sequence of 2137-3360 of SEQ ID NO:3 (corresponding to exons 16-25 of the a KIF5B gene) or a fragment thereof, or a sequence substantially identical thereto. In yet other examples, the nucleic acid molecule includes the nucleotide sequence shown in FIGs. 4A-4D (*e.g.*, SEQ ID NO:3) or a fragment thereof, or a sequence substantially identical thereto. In one example, the nucleic acid molecule is complementary to at least a portion of a nucleotide sequence disclosed herein, *e.g.*, is capable of hybridizing under a stringency condition described herein to SEQ ID NO:3 or a fragment thereof. In yet other example, the nucleic acid molecule hybridizes to a nucleotide sequence that is complementary to at least a portion of a nucleotide sequence disclosed herein, *e.g.*, is capable of hybridizing under a stringency condition to a nucleotide sequence complementary to SEQ ID NO:3 or a fragment thereof. The nucleotide sequence of a cDNA encoding an exemplary 5'RET-3'KIF5B fusion is shown in SEQ ID NO:3, and the predicted amino acid sequence is shown in SEQ ID NO:4. RT-PCR studies have shown that the RET exon 11 and KIF5B exon 16 did not yield a transcription product.

In a related example, the disclosure features nucleic acid constructs that include the KIF5B-RET nucleic acid molecules described herein. In certain examples, the nucleic acid molecules are operatively linked to a native or a heterologous regulatory sequence. Also included are vectors and host cells that include the KIF5B-RET nucleic acid molecules described herein, *e.g.*, vectors and host cells suitable for producing the nucleic acid molecules and polypeptides described herein.

In a related example, methods of producing the nucleic acid molecules and polypeptides described herein are also described.

In another example, the disclosure features nucleic acid molecules that reduces or inhibits the expression of a nucleic acid molecule that encodes a KIF5B-RET fusion described herein. Examples of such nucleic acid molecules include, for example, antisense molecules, ribozymes, RNAi, triple helix molecules that hybridize to a nucleic

acid encoding KIF5B-RET, or a transcription regulatory region of KIF5B-RET, and blocks or reduces mRNA expression of KIF5B-RET.

Nucleic Acid Detection and Capturing Reagents

The disclosure also features a nucleic acid molecule, *e.g.*, nucleic acid fragment, suitable as probe, primer, bait or library member that includes, flanks, hybridizes to, which are useful for identifying, or are otherwise based on, the KIF5B-RET fusions described herein. In certain examples, the probe, primer or bait molecule is an oligonucleotide that allows capture, detection or isolation of a KIF5B-RET fusion nucleic acid molecule described herein. The oligonucleotide can comprise a nucleotide sequence substantially complementary to a fragment of the KIF5B-RET fusion nucleic acid molecules described herein. The sequence identity between the nucleic acid fragment, *e.g.*, the oligonucleotide, and the target KIF5B-RET sequence need not be exact, so long as the sequences are sufficiently complementary to allow the capture, detection or isolation of the target sequence. In one example, the nucleic acid fragment is a probe or primer that includes an oligonucleotide between about 5 and 25, *e.g.*, between 10 and 20, or 10 and 15 nucleotides in length. In other examples, the nucleic acid fragment is a bait that includes an oligonucleotide between about 100 to 300 nucleotides, 130 and 230 nucleotides, or 150 and 200 nucleotides, in length.

In one example, the nucleic acid fragment can be used to identify or capture, *e.g.*, by hybridization, a KIF5B-RET fusion. For example, the nucleic acid fragment can be a probe, a primer, or a bait, for use in identifying or capturing, *e.g.*, by hybridization, a KIF5B-RET fusion described herein. In one example, the nucleic acid fragment can be useful for identifying or capturing a KIF5B-RET breakpoint, *e.g.*, as identified in FIG. 1. In one example, the nucleic acid fragment hybridizes to a nucleotide sequence within a chromosomal rearrangement that creates an in-frame fusion of exon 15 of KIF5B with exon 12 of RET (*e.g.*, a sequence within an 11MB pericentric inversion on chromosome 10). In one example, the nucleic acid fragment hybridizes to a nucleotide sequence in the region of 32,316,376-32,316,416 of chromosome 10 coupled to (*e.g.,* juxtaposed to) nucleotides in the region of nucleotides 43,611,042-43,611,118 of chromosome 10. In one example, the nucleic acid fragment hybridizes to a nucleotide sequence that includes a breakpoint, *e.g.*, a breakpoint as identified in FIGs. 1,

2 and 3A-3D. For example, the nucleic acid fragment can hybridize to a nucleotide sequence that includes the fusion junction between the KIF5B transcript and the RET transcript, *e.g.*, a nucleotide sequence that includes a portion of SEQ ID NO:1 (*e.g.*, a nucleotide sequence within exons 1-15 of a KIF5B gene and 12-20 of a RET gene) (*e.g.*, a portion of SEQ ID NO:1 comprising nucleotides 1720-1731, 1717-1734, or 1714-1737 of SEQ ID NO:1 (see FIG. 3B)).

The probes or primers described herein can be used, for example, for FISH detection or PCR amplification. In one exemplary example where detection is based on PCR, amplification of the KIF5B-RET fusion junction can be performed using a primer or a primer pair, *e.g.*, for amplifying a sequence flanking the KIF5B-RET fusion junctions described herein, *e.g.*, the mutations or the junction of a chromosomal rearrangement described herein. In one example, a pair of isolated oligonucleotide primers can amplify a region containing or adjacent to a position in the KIF5B-RET fusion. For example, forward primers can be designed to hybridize to a nucleotide sequence within KIF5B genomic or mRNA sequence (*e.g.*, a nucleotide sequence within exons 1-15 of a KIF5B gene, or nucleotides 1-1725 of SEQ ID NO:1), and the reverse primers can be designed to hybridize to a nucleotide sequence within RET (*e.g.*, a nucleotide sequence within exons 12-20 of RET, or nucleotides 1725-2934 of SEQ ID NO:1).

In another example, the nucleic acid fragments can be used to identify, *e.g.*, by hybridization, a 5'RET-3'KIF5B fusion. In one example, the nucleic acid fragment hybridizes to a nucleotide sequence that include a fusion junction between the RET transcript and the KIF5B transcript, *e.g.*, a nucleotide sequence within SEQ ID NO:3 (*e.g.*, a sequence comprising nucleotides 2131-2142, 2128-2145, or 2125-2148 of SEQ ID NO:3 (see FIG. 4B)).

In other examples, the nucleic acid fragment includes a bait that comprises a nucleotide sequence that hybridizes to a KIF5B-RET fusion nucleic acid molecule described herein, and thereby allows the capture or isolation said nucleic acid molecule. In one example, a bait is suitable for solution phase hybridization. In other examples, a bait includes a binding entity, *e.g.*, an affinity tag, that allows capture and separation, *e.g.*, by binding to a binding entity, of a hybrid formed by a bait and a nucleic acid hybridized to the bait.

In other examples, the nucleic acid fragment includes a library member comprising a KIF5B-RET nucleic acid molecule described herein. In one example, the library member includes a rearrangement that results in a KIF5B-RET fusion described herein.

The nucleic acid fragment can be detectably labeled with, *e.g.,* a radiolabel, a fluorescent label, a bioluminescent label, a chemiluminescent label, an enzyme label, a binding pair label, or can include an affinity tag; a tag, or identifier (*e.g*., an adaptor, barcode or other sequence identifier).

KIF5B-RET Fusion Polypeptides

In another example, the disclosure features a KIF5B-RET fusion polypeptide (*e.g*., a purified KIF5B-RET fusion polypeptide), a biologically active or antigenic fragment thereof, as well as reagents (*e.g*., antibody molecules that bind to a KIF5B-RET fusion polypeptide), methods for modulating a KIF5B-RET polypeptide activity and detection of a KIF5B-RET polypeptide.

In one example, the KIF5B-RET fusion polypeptide has at least one biological activity, *e.g*., a RET kinase activity, and/or a dimerizing or multimerizing activity. In one example, at least one biological activity of the KIF5B-RET fusion polypeptide is reduced or inhibited by an anti-cancer drug, *e.g*., a kinase inhibitor (*e.g*., a multikinase inhibitor or a RET-specific inhibitor). In one example, at least one biological activity of the KIF5B-RET fusion polypeptide is reduced or inhibited by a kinase inhibitor chosen from lenvatinib (E7080), sorafenib (NEXAVAR®), sunitinib (SUTENT®, SU11248), vandetanib (CAPRELSA®, ZACTIMA®, ZD6474), NVP-AST487, regorafenib (BAY-73-4506), motesanib (AMG 706), cabozantinib (XL-184), apatinib (YN-968D1), or DCC-2157.

In other examples, the KIF5B-RET fusion polypeptide includes a fragment of a KIF5B polypeptide and a fragment of a RET polypeptide. In one example, the KIF5B-RET fusion polypeptide includes amino acids 578-575 of SEQ ID NO:2 or a fragment thereof (*e.g*., amino acids 1-575 of SEQ ID NO:2 or a fragment thereof), and amino acids 576-624 of SEQ ID NO:2 or a fragment thereof (*e.g*., amino acids 576-977 of SEQ ID NO:2 or a fragment thereof). In yet other examples, the KIF5B-RET fusion polypeptide includes an amino acid sequence substantially identical to an in-frame

fusion of amino acids 578-575 of SEQ ID NO:2 or a fragment thereof (*e.g.*, amino acids 1-575 of SEQ ID NO:2 or a fragment thereof), and amino acids 576-624 of SEQ ID NO:2 or a fragment thereof (*e.g.*, amino acids 576-977 of SEQ ID NO:2 or a fragment thereof).

In other examples, the KIF5B-RET fusion polypeptide includes a KIF5B kinesin motor domain or a fragment thereof, and a RET kinase domain or a fragment thereof. In another example, the KIF5B-RET fusion polypeptide includes the amino acid sequence of amino acids 1-575 of SEQ ID NO:2 or a fragment thereof, or a sequence substantially identical thereto. For example, the KIF5B-RET fusion polypeptide can include a kinesin motor domain of KIF5B that includes amino acids 6-325 of SEQ ID NO:2 or a fragment thereof. In other examples, the KIF5B-RET fusion polypeptide includes the amino acid sequence of amino acids 576-977 of SEQ ID NO:2 or a fragment thereof, or a sequence substantially identical thereto. For example, the KIF5B-RET fusion polypeptide can include a RET kinase domain that includes amino acids 587-868 of SEQ ID NO:2 or a fragment thereof. In yet other examples, the KIF5B-RET fusion polypeptide includes the amino acid sequence shown in FIGs. 3A-3D (*e.g.*, SEQ ID NO:2) or a fragment thereof, or a sequence substantially identical thereto.

In yet other examples, the KIF5B-RET fusion polypeptide is encoded by a nucleic acid molecule described herein. In one example, the KIF5B-RET fusion polypeptide is encoded by an in-frame fusion of exon 15 of KIF5B with exon 12 of RET (*e.g.*, a sequence within an 11MB pericentric inversion on chromosome 10). In other examples, the KIF5B-RET fusion polypeptide is encoded by a nucleotide sequence in the region of 32,316,376-32,316,416 of chromosome 10 coupled to (*e.g.,* juxtaposed to) nucleotides in the region of nucleotides 43,611,042-43,611,118 of chromosome 10. In another example, the KIF5B-RET fusion polypeptide includes an amino acid sequence encoded by a nucleotide sequence comprising a fusion junction between the KIF5B transcript and the RET transcript, *e.g.*, a nucleotide sequence that includes a portion of SEQ ID NO:1 (*e.g.*, a nucleotide sequence within exons 1-15 of a KIF5B gene and 12-20 of a RET gene) (*e.g.*, a portion of SEQ ID NO:1 comprising nucleotides 1720-1731, 1717-1734, or 1714-1737 of SEQ ID NO:1 (see FIG. 3B)).

In yet other examples, the KIF5B-RET fusion polypeptide is encoded by a 5'-RET to 3'- KIF5B nucleic acid molecule described herein. In one example, the KIF5B-RET fusion polypeptide is encoded by a nucleotide sequence that include a fusion

junction between the RET transcript and the KIF5B transcript, *e.g.*, a nucleotide sequence within SEQ ID NO:3 (*e.g.*, a sequence comprising nucleotides 2131-2142, 2128-2145, or 2125-2148 of SEQ ID NO:3 (see FIG. 4B)). In yet other examples, the KIF5B-RET fusion polypeptide is encoded by the nucleotide sequence shown in FIGs. 4A-4D (*e.g.*, SEQ ID NO:3) or a fragment thereof, or a sequence substantially identical thereto.

In an example, the 5'KIF5B-3'RET fusion polypeptide comprises sufficient KIF5B and sufficient RET sequence such that it has kinase activity, *e.g.*, has elevated activity, *e.g.*, kinase activity, as compared with wild type RET, e.g., in a cell of a cancer referred to herein. In an example the 5'KIF5B-3'RET fusion polypeptide comprises at least 1, 2, 3, 4, 5, 6, 7, 9, 10, or 11 exons from KIF5B and at least 1, 2, 3, 4, 5, 6, 7, 9, or 10, RET exons. In one example, the 5'KIF5B-3'RET fusion polypeptide includes a kinesin motor domain or a functional fragment thereof, and a RET tyrosine kinase domain or a functional fragment thereof. In a related example, the disclosure features KIF5B-RET fusion polypeptide or fragments operatively linked to heterologous polypeptides to form fusion proteins.

In another example, the KIF5B-RET fusion polypeptide or fragment is a peptide, *e.g.*, an immunogenic peptide or protein, that contains a fusion junction described herein. Such immunogenic peptides or proteins can be used to raise antibodies specific to the fusion protein. In other examples, such immunogenic peptides or proteins can be used for vaccine preparation. The vaccine preparation can include other components, *e.g.*, an adjuvant.

In another example, the disclosure features antibody molecules that bind to a KIF5B-RET fusion polypeptide or fragment described herein. In examples the antibody can distinguish wild type RET (or KIF5B) from KIF5B-RET.

Methods Reducing a KIF5B-RET Activity

In another example, the disclosure features a method of reducing an activity of a KIF5B-RET fusion. The method includes contacting the KIF5B-RET fusion, or a KIF5B-RET-expressing cell, with an agent that inhibits an activity or expression of KIF5B-RET (*e.g.*, a kinase inhibitor). In one example, the contacting step can be effected *in vitro*, *e.g.*, in a cell lysate or in a reconstituted system. Alternatively, the method can be performed on cells in culture, *e.g.*, *in vitro* or *ex vivo*. In other

examples, the method can be performed on KIF5B-RET-expressing cells present in a subject, *e.g.*, as part of an *in vivo* (e.g., therapeutic or prophylactic) protocol. In an example the method is practiced on an animal subject (*e.g.*, an *in vivo* animal model). In certain examples, the KIF5B-RET fusion is a nucleic acid molecule or a polypeptide as described herein.

In a related example, a method of inhibiting, reducing, or treating a hyperproliferative disorder, *e.g.*, a cancer, in a subject is provided. The method includes administering to the subject a preselected therapeutic agent, *e.g.*, an anti-cancer agent (*e.g.*, a kinase inhibitor), as a single agent, or in combination, in an amount sufficient to reduce, inhibit or treat the activity or expression of KIF5B-RET (*e.g.*, a KIF5B-RET fusion described herein), thereby inhibiting, reducing, or treating the hyperproliferative disorder in the subject. "Treatment" as used herein includes, but is not limited to, inhibiting tumor growth, reducing tumor mass, reducing size or number of metastatic lesions, inhibiting the development of new metastatic lesions, prolonged survival, prolonged progression-free survival, prolonged time to progression, and/or enhanced quality of life.

In one example, the kinase inhibitor is administered based on a determination that a KIF5B-RET fusion is present in a subject, e.g., based on its present in a subject's sample. Thus, treatment can be combined with a KIF5B-RET detection or evaluation method, *e.g.*, as described herein, or administered in response to a determination made by a KIF5B-RET detection or evaluation method, e.g., as described herein. In certain examples, the kinase inhibitor is administered responsive to acquiring knowledge or information of the presence of the KIF5B-RET fusion in a subject. In one example, the kinase inhibitor is administered responsive to acquiring knowledge or information on the subject's genotype, *e.g.*, acquiring knowledge or information that the patient's genotype has a KIF5B-RET fusion. In other examples, the kinase inhibitor is administered responsive to receiving a communication (*e.g.*, a report) of the presence of the KIF5B-RET fusion in a subject (*e.g.*, a subject's sample). In yet other examples, the kinase inhibitor is administered responsive to information obtained from a collaboration with another party that identifies the presence of the KIF5B-RET fusion in a subject (*e.g.*, a subject's sample). In other examples, the kinase inhibitor is administered

responsive to a determination that the KIF5B-RET fusion is present in a subject. In one example, the determination of the presence of the KIF5B-RET fusion is carried out using one or more of the methods, *e.g.*, the sequencing methods, described herein. In other examples, the determination of the presence of the KIF5B-RET fusion includes receiving information on the subject's KIF5B-RET fusion genotype, *e.g.*, from another party or source.

The methods can, optionally, further include the step(s) of identifying (*e.g.*, evaluating, diagnosing, screening, and/or selecting) a subject at risk of having, or having, a KIF5B-RET fusion. In one example, the method further includes one or more of: acquiring knowledge or information of the presence of the KIF5B-RET fusion in a subject (*e.g.*, a subject's sample); acquiring knowledge or information on the subject's genotype, *e.g.*, acquiring knowledge or information that the patient's genotype has a KIF5B-RET fusion; receiving a communication (*e.g.*, a report) of the presence of the KIF5B-RET fusion in a subject (*e.g.*, a subject's sample); or collaborating with another party that identifies the presence of the KIF5B-RET fusion in a subject.

In one example, the subject treated has a KIF5B-RET fusion; *e.g.*, the subject has a tumor or cancer harboring a KIF5B-RET fusion. In other examples, the subject has been previously identified as having a KIF5B-RET fusion. In yet other examples, the subject has been previously identified as being likely or unlikely to respond to treatment with a protein kinase inhibitor, *e.g.*, a subject that has previously participated in a clinical trial. In other examples, the subject has been previously identified as being likely or unlikely to respond to treatment with a protein kinase inhibitor, based on the presence of the KIF5B-RET fusion. In one example, the subject is a mammal, *e.g.*, a human. In one example, the subject has, or at risk of having a cancer at any stage of disease. In other examples, the subject is a patient, *e.g.*, a cancer patient.

In other examples, the subject treated is a cancer patient who has participated in a clinical trial. For example, the subject participated in a clinical trial that evaluated a kinase inhibitor (*e.g.*, a multikinase inhibitor, a RET kinase inhibitor). In other example, the subject participated in a clinical trial that evaluates upstream

or downstream targets of RET. In one example, said cancer patient responded to the kinase inhibitor evaluated.

In certain examples, the cancer is a solid tumor, a soft tissue tumor, or a metastatic lesion. In one example, the cancer is chosen from a lung cancer, a pancreatic cancer, melanoma, a colorectal cancer, an esophageal-gastric cancer, a thyroid cancer, or an adenocarcinoma. In other example, the lung cancer is chosen from one or more of the following: non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), squamous cell carcinoma (SCC), adenocarcinoma of the lung, bronchogenic carcinoma, or a combination thereof. In one example, the lung cancer is NSCLC or SCC.

In one example, the anti-cancer agent is a kinase inhibitor. For example, the kinase inhibitor is a multi-kinase inhibitor or a RET-specific inhibitor. In one example, the kinase inhibitor is chosen from lenvatinib (E7080) (Eisai Co.), sorafenib (NEXAVAR®), sunitinib (SUTENT®, SU11248), vandetanib (CAPRELSA®, ZACTIMA®, ZD6474), NVP-AST487, regorafenib (BAY-73-4506), motesanib (AMG 706), cabozantinib (XL-184), apatinib (YN-968D1), or DCC-2157.

In other examples, the anti-cancer agent is a KIF5B-RET antagonist inhibits the expression of nucleic acid encoding KIF5B-RET. Examples of such KIF5B-RET antagonists include nucleic acid molecules, for example, antisense molecules, ribozymes, RNAi, triple helix molecules that hybridize to a nucleic acid encoding KIF5B-RET, or a transcription regulatory region, and blocks or reduces mRNA expression of KIF5B-RET.

In other examples, the kinase inhibitor is administered in combination with a second therapeutic agent or a different therapeutic modality, *e.g.*, anti-cancer agents, and/or in combination with surgical and/or radiation procedures. For example, the second therapeutic agent can be a cytotoxic or a cytostatic agent. Exemplary cytotoxic agents include antimicrotubule agents, topoisomerase inhibitors, or taxanes, antimetabolites, mitotic inhibitors, alkylating agents, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis and radiation. In yet other examples, the methods can be used in combination with immunodulatory agents, *e.g.*, IL-1, 2, 4, 6, or 12, or interferon alpha or gamma, or immune cell growth factors such as GM-CSF.

Screening Methods

In another example, the disclosure features a method, or assay, for screening for agents that modulate, *e.g.*, inhibit, the expression or activity of a KIF5B-RET fusion, *e.g.*, a KIF5B-RET fusion as described herein. The method includes contacting a KIF5B-RET fusion, or a cell expressing a KIF5B-RET fusion, with a candidate agent; and detecting a change in a parameter associated with a KIF5B-RET fusion, *e.g.*, a change in the expression or an activity of the KIF5B-RET fusion. The method can, optionally, include comparing the treated parameter to a reference value, *e.g.*, a control sample (*e.g.*, comparing a parameter obtained from a sample with the candidate agent to a parameter obtained from a sample without the candidate agent). In one example, if a decrease in expression or activity of the KIF5B-RET fusion is detected, the candidate agent is identified as an inhibitor. In another example, if an increase in expression or activity of the KIF5B-RET fusion is detected, the candidate agent is identified as an activator. In certain examples, the KIF5B-RET fusion is a nucleic acid molecule or a polypeptide as described herein.

In one example, the contacting step is effected in a cell-free system, *e.g.,* a cell lysate or in a reconstituted system. In other examples, the contacting step is effected in a cell in culture, *e.g.*, a cell expressing a KIF5B-RET fusion (*e.g.*, a mammalian cell, a tumor cell or cell line, a recombinant cell). In yet other examples, the contacting step is effected in a cell *in vivo* (a KIF5B-RET-expressing cell present in a subject, *e.g.*, an animal subject (*e.g.*, an *in vivo* animal model).

Exemplary parameters evaluated include one or more of:

(i) a change in binding activity, *e.g.*, direct binding of the candidate agent to a KIF5B-RET fusion polypeptide; a binding competition between a known ligand and the candidate agent to a KIF5B-RET fusion polypeptide;

(ii) a change in kinase activity, *e.g.*, phosphorylation levels of a KIF5B-RET fusion polypeptide (*e.g.,* an increased or decreased autophosphorylation); or a change in phosphorylation of a target of a RET kinase, *e.g.*, focal adhesion kinase (FAK), persephin or glial derived neurotrophic factor (GDNF), In certain examples, a change in kinase activity, *e.g.*, phosphorylation, is detected by any of Western blot (*e.g.*, using an anti-KIF5B or anti-RET antibody; a phosphor-specific antibody, detecting a shift in the

molecular weight of a KIF5B-RET fusion polypeptide), mass spectrometry, immunoprecipitation, immunohistochemistry, immunomagnetic beads, among others;

(iii) a change in an activity of a cell containing a KIF5B-RET fusion (*e.g.*, a tumor cell or a recombinant cell), *e.g.*, a change in proliferation, morphology or tumorigenicity of the cell;

(iv) a change in tumor present in an animal subject, *e.g.*, size, appearance, proliferation, of the tumor; or

(v) a change in the level, *e.g.*, expression level, of a KIF5B-RET fusion polypeptide or nucleic acid molecule.

In one example, a change in a cell free assay in the presence of a candidate agent is evaluated. For example, an activity of a KIF5B-RET fusion, or interaction of a KIF5B-RET fusion with a downstream ligand can be detected. In one example, a KIF5B-RET fusion polypeptide is contacted with a ligand, *e.g.*, in solution, and a candidate agent is monitored for an ability to modulate, *e.g.*, inhibit, an interaction, *e.g.*, binding, between the KIF5B-RET fusion polypeptide and the ligand.

In other examples, a change in an activity of a cell is detected in a cell in culture, *e.g.*, a cell expressing a KIF5B-RET fusion (*e.g.*, a mammalian cell, a tumor cell or cell line, a recombinant cell). In one example, the cell is a recombinant cell that is modified to express a KIF5B-RET fusion nucleic acid, *e.g.*, is a recombinant cell transfected with a KIF5B-RET fusion nucleic acid. The transfected cell can show a change in response to the expressed KIF5B-RET fusion, *e.g.*, increased proliferation, changes in morphology, increased tumorigenicity, and/or acquired a transformed phenotype. A change in any of the activities of the cell, *e.g.*, the recombinant cell, in the presence of the candidate agent can be detected. For example, a decrease in one or more of: proliferation, tumorigenicity, transformed morphology, in the presence of the candidate agent can be indicative of an inhibitor of a KIF5B-RET fusion. In other examples, a change in binding activity or phosphorylation as described herein is detected.

In yet other example, a change in a tumor present in an animal subject (*e.g.*, an *in vivo* animal model) is detected. In one example, the animal model is a tumor containing animal or a xenograft comprising cells expressing a KIF5B-RET fusion (*e.g.*, tumorigenic cells expressing a KIF5B-RET fusion). The candidate agent can be

administered to the animal subject and a change in the tumor is detected. In one example, the change in the tumor includes one or more of a tumor growth, tumor size, tumor burden, survival, is evaluated. A decrease in one or more of tumor growth, tumor size, tumor burden, or an increased survival is indicative that the candidate agent is an inhibitor.

In other examples, a change in expression of a KIF5B-RET fusion can be monitored by detecting the nucleic acid or protein levels, *e.g.*, using the methods described herein.

In certain examples, the screening methods described herein can be repeated and/or combined. In one example, a candidate agent that is evaluated in a cell-free or cell-based described herein can be further tested in an animal subject.

In one example, the candidate agent is a small molecule compound, e.g., a kinase inhibitor, a nucleic acid (e.g., antisense, siRNA, aptamer, ribozymes, microRNA), an antibody molecule (*e.g.,* a full antibody or antigen binding fragment thereof that binds to KIF5B5 or RET). The candidate agent can be obtained from a library (*e.g.*, a commercial library of kinase inhibitors) or rationally designed (*e.g.*, based on the RET kinase domain).

Methods for Detecting KIF5B-RET Fusions

In another example, the disclosure features a method of determining the presence of a KIF5B-RET fusion, *e.g.*, a KIF5B-RET fusion as described herein. In one example, the KIF5B-RET fusion is detected in a nucleic acid molecule or a polypeptide. The method includes detecting whether a KIF5B-RET fusion nucleic acid molecule or polypeptide is present in a cell (*e.g.,* a circulating cell), a tissue (*e.g.*, a tumor), or a sample, e.g., a tumor sample, from a subject. In one example, the sample is a nucleic acid sample. In one example, the nucleic acid sample comprises DNA, *e.g.*, genomic DNA or cDNA, or RNA, *e.g.*, mRNA. In other examples, the sample is a protein sample.

In one example, the sample is, or has been, classified as non-malignant using other diagnostic techniques, e.g., immunohistochemistry.

In one example, the sample is acquired from a subject (e.g., a subject having or at risk of having a cancer, *e.g.*, a patient), or alternatively, the method further includes

acquiring a sample from the subject. The sample can be chosen from one or more of: tissue, e.g., cancerous tissue (*e.g.*, a tissue biopsy), whole blood, serum, plasma, buccal scrape, sputum, saliva, cerebrospinal fluid, urine, stool, circulating tumor cells, circulating nucleic acids, or bone marrow. In certain examples, the sample is a tissue (*e.g.*, a tumor biopsy), a circulating tumor cell or nucleic acid.

In examples, the tumor is from a cancer described herein, *e.g.*, is chosen from a lung cancer, a colorectal cancer, an esophageal-gastric cancer, a thyroid cancer, an adenocarcinoma or a melanoma. In one example, the tumor is from a lung cancer, *e.g.*, a NSCLC, a SCLC, a SCC, or a combination thereof.

In one example, the subject is at risk of having, or has a cancer (*e.g.*, a patient with a cancer described herein). For example, in one example, the subject is at risk for having, or has a lung cancer.

In other examples, the KIF5B-RET fusion is detected in a nucleic acid molecule by a method chosen from one or more of: nucleic acid hybridization assay, amplification-based assays (*e.g.*, polymerase chain reaction (PCR)), PCR-RFLP assay, real-time PCR, sequencing, screening analysis (including metaphase cytogenetic analysis by standard karyotype methods, FISH (*e.g.*, break away FISH), spectral karyotyping or MFISH, comparative genomic hybridization), *in situ* hybridization, SSP, HPLC or mass-spectrometric genotyping.

In one example, the method includes: contacting a nucleic acid sample, *e.g.*, a genomic DNA sample (*e.g.*, a chromosomal sample or a fractionated, enriched or otherwise pre-treated sample) or a gene product (mRNA, cDNA), obtained from the subject, with a nucleic acid fragment (*e.g.*, a probe or primer as described herein (*e.g.*, an exon-specific probe or primer) under conditions suitable for hybridization, and determining the presence or absence of the KIF5B-RET nucleic acid molecule. The method can, optionally, include enriching a sample for the gene or gene product.

In a related example, a method for determining the presence of a KIF5B-RET fusion nucleic acid molecule is provided. The method includes: acquiring a sequence for a position in a nucleic acid molecule, *e.g.*, by sequencing at least one nucleotide of the nucleic acid molecule (*e.g.*, sequencing at least one nucleotide in the nucleic acid molecule that comprises the KIF5B-RET fusion), thereby determining that the KIF5B-RET fusion is present in the nucleic acid molecule. Optionally, the sequence acquired is

compared to a reference sequence, or a wild type reference sequence. In one example, the nucleic acid molecule is from a cell (*e.g.,* a circulating cell), a tissue (*e.g*., a tumor), or any sample from a subject (*e.g*., blood or plasma sample). In other examples, the nucleic acid molecule from a tumor sample (*e.g*., a tumor or cancer sample) is sequenced. In one example, the sequence is determined by a next generation sequencing method. The method further can further include acquiring, *e.g*., directly or indirectly acquiring, a sample, *e.g*., a tumor or cancer sample, from a subject (*e.g*., a patient). In certain examples, the cancer is chosen from a lung cancer, colorectal cancer, esophageal-gastric cancer or melanoma.

In another example, the disclosure features a method of analyzing a tumor or a circulating tumor cell. The method includes acquiring a nucleic acid sample from the tumor or the circulating cell; and sequencing, *e.g*., by a next generation sequencing method, a nucleic acid molecule, *e.g*., a nucleic acid molecule that includes a KIF5B-RET fusion described herein.

In yet another example, a KIF5B-RET fusion polypeptide is detected. The method includes: contacting a protein sample with a reagent which specifically binds to a KIF5B-RET fusion polypeptide; and detecting the formation of a complex of the KIF5B-RET fusion polypeptide and the reagent. In one example, the reagent is labeled with a detectable group to facilitate detection of the bound and unbound reagent. In one example, the reagent is an antibody molecule, *e.g*., is selected from the group consisting of an antibody, and antibody derivative, and an antibody fragment.

In yet another example, the level (*e.g.,* expression level) or activity the KIF5B-RET fusion is evaluated. For example, the level (*e.g.,* expression level) or activity of the KIF5B-RET fusion (*e.g*., mRNA or polypeptide) is detected and (optionally) compared to a pre-determined value, *e.g*., a reference value (*e.g*., a control sample).

In yet another example, the KIF5B-RET fusion is detected prior to initiating, during, or after, a treatment in a subject, *e.g*., a treatment with a kinase inhibitor. In one example, the KIF5B-RET fusion is detected at the time of diagnosis with a cancer. In other example, the KIF5B-RET fusion is detected at a pre-determined interval, *e.g*., a first point in time and at least at a subsequent point in time.

In certain examples, responsive to a determination of the presence of the KIF5B-RET fusion, the method further includes one or more of:

(1) stratifying a patient population (*e.g.*, assigning a subject, *e.g.*, a patient, to a group or class);

(2) identifying or selecting the subject as likely or unlikely to respond to a treatment, *e.g.*, a kinase inhibitor treatment as described herein;

(3) selecting a treatment option, *e.g.,* administering or not administering a preselected therapeutic agent, *e.g.*, a kinase inhibitor as described herein; or

(4) prognosticating the time course of the disease in the subject (*e.g.*, evaluating the likelihood of increased or decreased patient survival).

In certain examples, the kinase inhibitor is a multi-kinase inhibitor or a RET-specific inhibitor. In one example, the kinase inhibitor is chosen from lenvatinib (E7080), sorafenib (NEXAVAR®), sunitinib (SUTENT®, SU11248), vandetanib (CAPRELSA®, ZACTIMA®, ZD6474), NVP-AST487, regorafenib (BAY-73-4506), motesanib (AMG 706), cabozantinib (XL-184), apatinib (YN-968D1), or DCC-2157.

In certain examples, responsive to the determination of the presence of the KIF5B-RET fusion, the subject is classified as a candidate to receive treatment with a kinase inhibitor, *e.g.*, a kinase inhibitor as described herein. In one example, responsive to the determination of the presence of the KIF5B-RET fusion, the subject, *e.g.*, a patient, can further be assigned to a particular class if a KIF5B-RET fusion is identified in a sample of the patient. For example, a patient identified as having a KIF5B-RET fusion can be classified as a candidate to receive treatment with a kinase inhibitor, *e.g.*, a kinase inhibitor as described herein. In one example, the subject, *e.g.*, a patient, is assigned to a second class if the mutation is not present. For example, a patient who has a lung tumor that does not contain a KIF5B-RET fusion, may be determined as not being a candidate to receive a kinase inhibitor, *e.g.*, a kinase inhibitor as described herein.

In another example, responsive to the determination of the presence of the KIF5B-RET fusion, the subject is identified as likely to respond to a treatment that comprises a kinase inhibitor *e.g.*, a kinase inhibitor as described herein.

In yet another example, responsive to the determination of the presence of the KIF5B-RET fusion, the method includes administering a kinase inhibitor, *e.g.*, a kinase inhibitor as described herein, to the subject.

In one aspect, the present disclosure refers to a method of determining the presence of a Kinesin Family Member 5B - RET proto-oncogene (KIF5B-RET) fusion, comprising acquiring knowledge by sequencing, e.g., next-generation sequencing (NGS) or sequencing by mass spectrometry, that a nucleic acid molecule encoding the KIF5B-RET fusion or a KIF5B-RET fusion polypeptide is present in a sample from a subject having cancer, wherein the cancer is chosen from a colorectal cancer or a lung cancer, optionally wherein the lung cancer is a NSCLC, a SCLC, a SCC, or a combination thereof, and wherein responsive to the acquisition of knowledge of the presence of the KIF5B-RET fusion:

(i) the subject is classified as a candidate to receive treatment with an anti-cancer agent; and/or

(ii) the subject is identified as likely to respond to a treatment that comprises an anti-cancer agent, wherein the anti-cancer agent is selected from:

(a) a multi-kinase inhibitor or a RET-specific inhibitor; or

(b) a nucleic acid molecule selected from the group consisting of antisense molecules, ribozymes, siRNA, and triple helix molecules, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule encoding the KIF5B-RET fusion, thereby blocking or reducing mRNA expression of the nucleic acid molecule encoding the KIF5B-RET fusion,

said method optionally further comprising providing a report to another party, wherein said report comprises:

information on the role of a KIF5B-RET fusion, or wildtype sequence, in disease;

information on prognosis, resistance, or potential or suggested therapeutic options for the subject;

information on the likely effectiveness of a therapeutic option, the acceptability of a therapeutic option, or the advisability of applying a therapeutic option to the subject; or

information, or a recommendation on, the administration of a drug to the subject.

Method of Evaluating a Tumor or a Subject

In another example, the disclosure features a method of evaluating a subject (*e.g.*, a patient), *e.g.*, for risk of having or developing a cancer, *e.g.*, a lung cancer, colorectal cancer or skin cancer. The method includes: acquiring information or knowledge of the presence of a KIF5B-RET fusion in a subject (*e.g.*, acquiring genotype information of the subject that identifies a KIF5B-RET fusion as being present in the subject); acquiring a sequence for a nucleic acid molecule identified herein (*e.g.*, a nucleic acid molecule that includes a KIF5B-RET fusion sequence); or detecting the presence of a KIF5B-RET fusion nucleic acid or polypeptide in the subject), wherein the presence of the KIF5B-RET fusion is positively correlated with increased risk for, or having, a cancer associated with the KIF5B-RET fusion.

The method can further include the step(s) of identifying (*e.g.*, evaluating, diagnosing, screening, and/or selecting) the subject as being positively correlated with increased risk for, or having, a cancer associated with the KIF5B-RET fusion. In one example, the subject is identified or selected as likely or unlikely to respond to a treatment, *e.g.*, a kinase inhibitor treatment as described herein.

The method can further include treating the subject with a kinase inhibitor, *e.g.*, a kinase inhibitor as described herein.

The method can further include acquiring, *e.g.*, directly or indirectly, a sample from a patient and evaluating the sample for the present of a KIF5B-RET fusion as described herein.

In certain examples, the subject is a patient or patient population that has participated in a clinical trial. In one example, the subject has participated in a clinical trial for evaluating a kinase inhibitor (*e.g.*, a multikinase inhibitor or a RET inhibitor). In one example, the clinical trial is discontinued or terminated. In other examples, the subject has participated in a clinical trial that evaluates a RET kinase, a KIF5B inhibitor (*e.g.*, a kinesin inhibitor), an upstream or downstream component of RET or KIF5B. In one example, the subject responded favorably to the clinical trial,

e.g., experience an improvement in at least one symptom of a cancer (*e.g.*, decreased in tumor size, rate of tumor growth, increased survival). In other examples, the subject did not respond in a detectable way to the clinical trial.

In a related example, a method of evaluating a patient or a patient population is provided. The method includes: identifying, selecting, or obtaining information or knowledge that the patient or patient population has participated in a clinical trial;

acquiring information or knowledge of the presence of a KIF5B-RET fusion in the patient or patient population (*e.g.*, acquiring genotype information of the subject that identifies a KIF5B-RET fusion as being present in the subject); acquiring a sequence for a nucleic acid molecule identified herein (*e.g.*, a nucleic acid molecule that includes a KIF5B-RET fusion sequence); or detecting the presence of a KIF5B-RET fusion nucleic acid or polypeptide in the subject), wherein the presence of the KIF5B-RET fusion is identifies the patient or patient population as having an increased risk for, or having, a cancer associated with the KIF5B-RET fusion.

In certain examples, the subject is a patient or patient population that has participated in a clinical trial. In one example, the subject has participated in a clinical trial for evaluating a kinase inhibitor (*e.g.*, a multikinase inhibitor or a RET inhibitor). In one example, the clinical trial is discontinued or terminated. In other examples, the subject has participated in a clinical trial that evaluates a RET kinase, a KIF5B inhibitor (*e.g.*, a kinesin inhibitor), an upstream or downstream component of RET or KIF5B. In one example, the subject responded favorably to the clinical trial, *e.g.*, experience an improvement in at least one symptom of a cancer (*e.g.*, decreased in tumor size, rate of tumor growth, increased survival). In other examples, the subject did not respond in a detectable way to the clinical trial.

In examples, the method further includes treating the subject with a kinase inhibitor, *e.g.*, a kinase inhibitor as described herein.

Reporting

Methods described herein can include providing a report, such as, in electronic, web-based, or paper form, to the patient or to another person or entity, *e.g.*, a caregiver, *e.g.*, a physician, *e.g.*, an oncologist, a hospital, clinic, third-party payor, insurance company or government office. The report can include output from the method, *e.g.*, the

identification of nucleotide values, the indication of presence or absence of a KIF5B-RET fusion as described herein, or wildtype sequence. In one example, a report is generated, such as in paper or electronic form, which identifies the presence or absence of an alteration described herein, and optionally includes an identifier for the patient from which the sequence was obtained.

The report can also include information on the role of a sequence, *e.g.*, a KIF5B-RET fusion as described herein, or wildtype sequence, in disease. Such information can include information on prognosis, resistance, or potential or suggested therapeutic options. The report can include information on the likely effectiveness of a therapeutic option, the acceptability of a therapeutic option, or the advisability of applying the therapeutic option to a patient, *e.g.*, a patient having a sequence, alteration or mutation identified in the test, and in examples, identified in the report. For example, the report can include information, or a recommendation on, the administration of a drug, *e.g.*, the administration at a preselected dosage or in a preselected treatment regimen, *e.g.*, in combination with other drugs, to the patient. In an example, not all mutations identified in the method are identified in the report. For example, the report can be limited to mutations in genes having a preselected level of correlation with the occurrence, prognosis, stage, or susceptibility of the cancer to treatment, *e.g.*, with a preselected therapeutic option. The report can be delivered, *e.g.*, to an entity described herein, within 7, 14, or 21 days from receipt of the sample by the entity practicing the method.

In another example, the disclosure features a method for generating a report, *e.g.*, a personalized cancer treatment report, by obtaining a sample, *e.g.*, a tumor sample, from a subject, detecting a KIF5B-RET fusion as described herein in the sample, and selecting a treatment based on the mutation identified. In one example, a report is generated that annotates the selected treatment, or that lists, *e.g.*, in order of preference, two or more treatment options based on the mutation identified. In another example, the subject, *e.g.*, a patient, is further administered the selected method of treatment.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the disclosure, suitable methods

and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and the example are illustrative only and not intended to be limiting.

The details of one or more examples featured in the disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages featured in the disclosure will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a snapshot of the sequencing reads illustrating that there are RET read pairs that map on one end to 5'KIF5B and on the other end to 3'RET, and other RET read pairs that map on one end to 5'RET and on the other end to 3'KIFB.

FIGs. 2A and 2B are schematic diagrams illustrating the inversion event that brings together exon 15 of KIF5B and exon 12 of RET.

FIGs. 3A to 3D are the predicted cDNA sequence (SEQ ID NO:1) and protein sequence of the KIF5B-RET fusion (SEQ ID NO:2). The KIF5B portion of the cDNA sequence is equivalent to nucleotides 471-2195 of RefSeq NM_004521.2, and the KIF5B portion of the protein sequence is equivalent to amino acids 1-575 of RefSeq NP_004512. The KIF5B portions of the cDNA and protein sequences are underlined. The RET portion of the cDNA sequence is equivalent to nucleotides 2327-3535 of RefSeq NM_020975, and the RET portion of the protein sequence is equivalent to amino acids 713-1114 of RefSeq NP_066124.1. The intact tyrosine kinase domain of RET is represented by a gray box (see FIG. 3C).

FIGs. 4A to 4D are the predicted cDNA sequence (SEQ ID NO:3) and protein sequence of the RET-KIF5B fusion (SEQ ID NO:4). The cDNA sequence of the RET portion of the fusion transcript is equivalent to nucleotides 190-2326 of RefSeq NM_020975, and the protein sequence is equivalent to amino acids 1-712 of RefSeq NP_066124.1. The cDNA sequence of the KIF5B portion of the fusion transcript is equivalent to nucleotides 2196-3362 of RefSeq NM_004521.2, and the protein portion is equivalent to amino acids 576-963 of RefSeq NP_004512. The KIF5B portions of the cDNA and protein sequences are underlined.

We Claim:

1. A method of determining the presence of a Kinesin Family Member 5B - RET proto-oncogene (KIF5B-RET) fusion, comprising acquiring knowledge by sequencing, e.g., next-generation sequencing (NGS) or sequencing by mass spectrometry, that a nucleic acid molecule encoding the KIF5B-RET fusion or a KIF5B-RET fusion polypeptide is present in a sample from a subject having cancer, wherein the cancer is chosen from a colorectal cancer or a lung cancer, optionally wherein the lung cancer is a NSCLC, a SCLC, a SCC, or a combination thereof, and wherein responsive to the acquisition of knowledge of the presence of the KIF5B-RET fusion:

(i) the subject is classified as a candidate to receive treatment with an anti-cancer agent; and/or

(ii) the subject is identified as likely to respond to a treatment that comprises an anti-cancer agent, wherein the anti-cancer agent is selected from:

(a) a multi-kinase inhibitor or a RET-specific inhibitor; or

(b) a nucleic acid molecule selected from the group consisting of antisense molecules, ribozymes, siRNA, and triple helix molecules, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule encoding the KIF5B-RET fusion, thereby blocking or reducing mRNA expression of the nucleic acid molecule encoding the KIF5B-RET fusion,

said method optionally further comprising providing a report to another party, wherein said report comprises:

information on the role of a KIF5B-RET fusion, or wildtype sequence, in disease;

information on prognosis, resistance, or potential or suggested therapeutic options for the subject;

information on the likely effectiveness of a therapeutic option, the acceptability of a therapeutic option, or the advisability of applying a therapeutic option to the subject; or

information, or a recommendation on, the administration of a drug to the subject.

2. The method of claim 1, wherein the sample:

(i) comprises fluid, cells, and/or a tissue, e.g., a tumor tissue;

(ii) is a nucleic acid sample or a protein sample;

(iii) is acquired from the subject;

(iv) comprises a tumor biopsy or a circulating tumor cell or nucleic acid; and/or

(v) is chosen from one or more of: tissue, e.g., cancerous tissue such as a tissue biopsy, whole blood, serum, plasma, buccal scrape, sputum, saliva, cerebrospinal fluid, urine, stool, circulating tumor cells, circulating nucleic acids, or bone marrow.

3. The method of claim 1, wherein a nucleic acid molecule encoding the KIF5B-RET fusion is determined to be present in a sample from the subject having cancer, and wherein the method further comprises a method chosen from one or more of: a nucleic acid hybridization assay, an amplification-based assay, a PCR-RFLP assay, real-time PCR, a screening analysis, FISH, spectral karyotyping or MFISH, comparative genomic hybridization, *in situ* hybridization, SSP, HPLC or mass-spectrometric genotyping.

4. The method of claim 1, wherein the method further comprises: contacting the sample with a reagent which specifically binds to a KIF5B-RET fusion polypeptide, wherein the sample is a protein sample; and detecting the formation of a complex of the KIF5B-RET fusion polypeptide and the reagent, optionally wherein: the reagent is labeled with a detectable group to facilitate detection of the bound and unbound reagent; and/or the reagent is an antibody molecule.

5. The method of claim 1, wherein:

(i) the level or activity of the KIF5B-RET fusion is evaluated;

(ii) the KIF5B-RET fusion is detected prior to initiating a treatment with the anti-cancer agent in the subject;

(iii) the KIF5B-RET fusion is detected at the time of diagnosis of the subject with a cancer; and/or

(iv) the KIF5B-RET fusion is detected at a pre-determined interval, e.g., a first point in time and at least at a subsequent point in time.

6. The method of claim 1, further comprising, responsive to a determination of the presence of the KIF5B-RET fusion, one or more of:

(i) stratifying a patient population;

(ii) identifying or selecting the subject as likely or unlikely to respond to a treatment;

(iii) selecting a treatment option for the subject, optionally wherein the treatment option comprises administering an anti-cancer agent chosen from lenvatinib, sorafenib, sunitinib, vandetanib, NVP-AST487, regorafenib, motesanib, cabozantinib, apatinib, or DCC-2157; or

(iv) prognosticating the time course of the cancer in the subject.

7. The method of claim 1, further comprising:

identifying, selecting, or obtaining information or knowledge that the subject has participated in a clinical trial or has been treated for cancer,

wherein the presence of the KIF5B-RET fusion identifies the subject as having an increased risk for, or having, a cancer associated with the KIF5B-RET fusion.