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Review

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Opportunities and challenges in developing tissue-agnostic anti-cancer drugs

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Abstract

The rapid advances in the understanding of oncogenic process and the maturation of affordable precision diagnostic tools have enabled the development of targeted therapeutic agents, such as those targeting BCR-ABL, epithelial growth factor receptor L858R, EML4-anaplastic lymphoma kinase, and BRAF V600E, to treat cancers that harbor specific molecular alterations. Traditionally, each targeted drug has been developed for a particular tumor type where such alteration is most frequently found. Recently, the widespread adoption of next generation sequencing has led to an increase in the identification of rare and ultra-rare alterations, and, in some cases, the same rare alterations are found across multiple tumor types. The rarity of these alterations makes clinical trials traditionally designed for specific tumor types infeasible. As a result, tissue-agnostic trials have been developed to study the efficacy of these treatments and increase patient access. This review summarizes current successful cases of tissue-agnostic development, such as drugs targeting tropomyosin receptor kinase fusions, and proposes the next wave of potential tissue-agnostic targets, including fusions of *ROS1*, anaplastic lymphoma kinase, fibroblast growth factor receptor, and rearranged during transfection. In addition, the advantages and the challenges of such approach are discussed in the context of clinical development and approval.

Keywords: Tissue agnostic, basket trial, tropomyosin receptor kinase, anaplastic lymphoma kinase, *ROS1*, fibroblast growth factor receptor, rearranged during transfection

INTRODUCTION

The past two decades have witnessed a significant paradigm shift in cancer treatment practices based on increased understanding of oncogenic process. Molecular alterations in specific targets, usually kinases, can result in constitutive activation of the targets and their downstream signaling activities, leading to unchecked cellular proliferation, resistance to cell death, promotion of angiogenesis, and evasion of immune surveillance, all of which are hallmarks of cancer[1]. Matching a patient's cancer with a therapeutic agent designed to specifically address the underlying molecular alteration has become the cornerstone of precision oncology[2]. Alongside the rapid advancement in cancer biology, the technical revolution of molecular diagnostic platforms, particularly high-throughput next generation sequencing (NGS), has made comprehensive profiling of tumor tissue and liquid biopsy samples feasible and affordable, not only for scientific interrogation of cancer genome, transcriptome, and epigenome for target discovery and mechanistic characterization, but also for patient selection and stratification in the clinical setting[3,4].

Several large-scale cancer sequencing efforts involving thousands of patient samples have not only confirmed relatively frequent molecular alterations such as mutations in Kirsten rat sarcoma gene (*KRas*), tumor protein p53 (TP53), and epithelial growth factor receptor (*EGFR*), but also revealed, in many cases for the first time, low- and ultra-low frequency mutations that otherwise had been difficult to detect without high-throughput deep sequencing in large number of samples. For instance, in a study by Armenia and colleagues[5], whole exome sequencing data from 1,013 cases of prostate cancer (680 primary and 333 metastatic tumors) and matched germline were assembled and uniformly analyzed. The study identified a total of 97 potential oncogenic genes, about 70 of which had not been previously implicated in the disease. The majority of these newly identified mutated genes were found in less than 5% of the 1,013 cases. In statistical terms, this is known as a "long-tail" distribution; in other words, some genes are mutated in comparatively many cases, but many genes with oncogenic mutations are only found in few cases. This "long tail" distribution also suggests that additional discovery of rarely mutated oncogenic drivers is likely to continue along with the dramatic increase in the number of tumors sequenced. A similar "long tail" distribution has also been observed in other tumor types, such as lung adenocarcinoma[6], head and neck[7], and breast[8]. Arguably, this "long tail" phenomenon exists in most, if not all, tumors. Interestingly, some of the same "long tail" genes are found across many distinct tumor types, suggesting common underlying mechanism of tumorigenesis[9,10].

The United States Food and Drug Administration (FDA) and other regulatory agencies generally approve anti-cancer drugs on the basis of efficacy and safety data obtained from clinical trials with patients of a particular tumor type. An example of this "one target, one tumor type" is the FDA's 2001 landmark approval of imatinib, a kinase inhibitor of Abelson tyrosine kinase (c-ABL), for the use in treating BCR-ABL positive, chronic myeloid leukemia (CML), which heralded a new era in approval of drugs for single indications with characteristic gene alterations[11]. A decade later, crizotinib, a small molecule tyrosine kinase inhibitor (TKI) of mesenchymal-epithelial transition factor (c-MET), anaplastic lymphoma kinase (ALK), c-ros oncogene 1 (ROS1), and recepteur d'Origine Nantais (RON), received accelerated approval for the treatment of patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) with EML4-ALK fusion. The approval was based on two single-arm trials demonstrating objective response rates (ORRs) of 50% and 61% and median response durations of 42 and 48 weeks[12].

Even with the life-changing success of the "one target, one tumor type" approach, it is important to remember that cancer is a complex disease. On the one hand, tumors that originate from the same tissue or organ can be segmented into multiple subtypes, each of which can be defined by differentiating molecular, pathological, and etiological features[13-15]. On the other hand, some distinct and seemingly unrelated tumors of different histology can be traced back to a common dominant genetic defect that can be exploited for therapeutic intervention by the same targeted agent, regardless of the histological tumor type

or anatomical site of origin[16]. Recently, drugs that target these common features, for example microsatellite instability-high (MSI-H)/mismatch repair deficient (dMMR)[17] and tropomyosin receptor kinase (TRK) fusions[18], across multiple tumor types have been approved by the FDA as the first wave of tissue-agnostic therapies (one target, all/many tumor types).

This review summarizes the current status of the tissue-agnostic approach and proposes additional molecular alterations, with the emphasis on oncogenic fusions, that are potential targets for drug discovery and development.

FIRST TISSUE-AGNOSTIC APPROVALS

The FDA and its international counterparts traditionally approve cancer drugs on the basis of clinical studies in patients of a particular tumor type. Even for biomarker-driven approvals such as erlotinib and crizotinib, these drugs have generally been approved for a specific tumor type that harbors the target of interest.

In 2017, however, a significant paradigm shift took place, when the FDA granted accelerated approval of pembrolizumab, an anti-programmed cell death protein 1 (PD-1) therapy, in adult and pediatric patients with locally advanced or metastatic solid tumors of any tumor type (hence, tissue agnostic) that are dMMR or MSI-H, who have progressed after prior treatment, and who have no satisfactory alternative treatment options[17]. This approval was based on collective data from several clinical trials. In the Phase II study code-named KEYNOTE-016 in patients (n = 58) with progressive metastatic carcinoma, high somatic mutation burden was associated with significant prolonged progression-free survival (PFS). In addition, two separate studies (KEYNOTE-158, n = 19; KEYNOTE-164, n = 61) specifically enrolled solid tumor patients with MSI-H or dMMR. Additional data from KEYNOTE-12 (n =6) and KEYNOTE-28 (n = 5) were included in the dataset after retrospective analysis of MSI and MMR status. By tumor type, among 149 patients with MSI-H and/or dMMR cancers across the five trials, the majority (n = 90) had colorectal cancer (CRC), while 14 other distinct tumor types accounted for the remaining 59 patients. Collectively, the ORR was 39.6%, which included 11 complete responses (CR) and 48 partial responses (PR). The response rate for patients with colorectal cancer and those with other cancers were similar.

About a year later in November 2018, the second tissue-agnostic cancer therapy, larotrectinib, won accelerated approval by the FDA for the treatment of adult and pediatric patients with solid tumors that have a neurotrophic receptor tyrosine kinase (*NTRK*) gene fusion without a known acquired resistance mutation, that are either metastatic or where surgical resection is likely to result in severe morbidity, and who have no satisfactory alternative treatments or whose cancer has progressed following treatment[19]. It is the second tissue-agnostic FDA approval for the treatment of cancer, and the first small molecule TKI that gained the tissue-agnostic status. The approval was based on clinical outcome in 55 patients with unresectable or metastatic, *NTRK*-fusion-positive solid tumors from three multicenter, open-label, single-arm clinical trials: LOXO-TRK-14001 (NCT02122913), SCOUT (NCT02637687), and NAVIGATE (NCT02576431). The identification of positive *NTRK* gene fusion status was prospectively determined in local laboratories using NGS or fluorescence in situ hybridization (FISH). The ORR was 75%, including 22% CR and 53% PR across 12 cancer types, with the most common being salivary gland tumors (22%), soft tissue sarcoma (20%), infantile fibrosarcoma (13%), and thyroid cancer (9%), as well as lung, melanoma, gastrointestinal stromal tumor (GIST), and colon cancer.

Soon after, the *TRK*/*ROS1* inhibitor, entrectinib, was also granted accelerated approval by the FDA for the treatment of adults and pediatric patients 12 years of age and older with solid tumors that have a *NTRK* gene fusion without a known acquired resistance mutation, are metastatic or where surgical resection is likely to result in severe morbidity, and have progressed following treatment or have no satisfactory

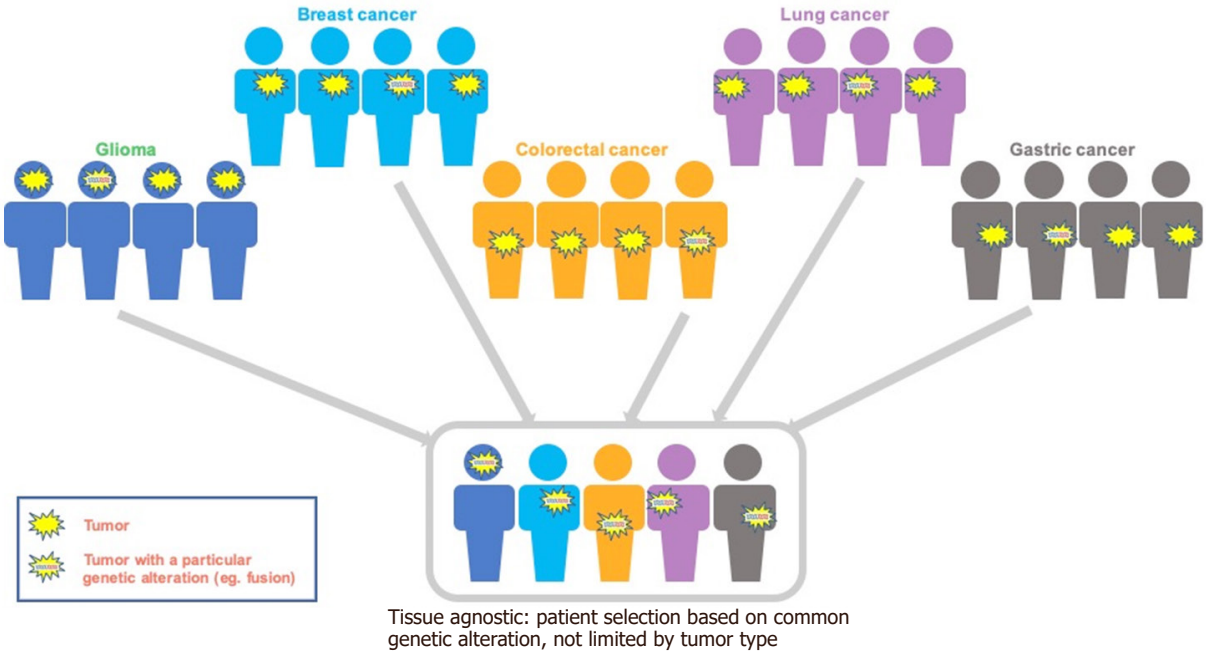

Figure 1. Tissue-agnostic approach offers biomarker-informed treatment strategy regardless of histological origin of the tumor

standard therapy. The approval was based on clinical outcome in 54 adult patients across three multicenter, single-arm, clinical trials: ALKA, STARTRK-1 (NCT02097810), and STARTRK-2 (NCT02568267). The identification of positive NTRK gene fusion status was determined in local laboratories or a central laboratory using nucleic acid-based tests prior to enrollment. Among 54 adult patients, the ORR was 57%, with 7.4% of patients achieving CR. The most common cancers were sarcoma, NSCLC, mammary analog secretory carcinoma, breast, thyroid, and colorectal. Compared to larotrectinib, the patient populations of entrectinib leaned more heavily on adult patients with more prior lines of therapies[20]. Importantly, entrectinib also showed meaningful responses in brain cancer patients and those whose tumors metastasized to the brain[21].

The approvals of these tissue-agnostic therapies represented a new paradigm in cancer treatment and validated the notion that, under certain circumstances, the biomarker in essence, rather than the tissue origin, would define the disease [Figure 1].

It is important to point out that the latest tissue-agnostic approvals are both small molecule receptor tyrosine kinase (RTK) inhibitors that treat oncogenic fusions in rare tumors. There are a number of novel compounds in development for other oncogenic fusion genes[22], and it is highly likely that next tissue-agnostic approval will be from one of these experimental agents.

ONCOGENIC GENE FUSIONS

In the past several decades, cancer epidemiological and molecular studies have identified a variety of genetic alterations including point mutations, chromosomal rearrangements and translocations, gene amplification, and overexpression that are believed to play a driver role in various cancer histologies[10]. Many of these changes lead to constitutive activation of the oncoprotein and downstream signaling pathways, resulting in uncontrolled cell proliferation, survival, and migration, which are hallmarks of cancer[23].

Oncogenic gene fusions are somatic genetic alteration caused by interchromosomal translocation, intrachromosomal translocation, insertion, deletion, tandem duplication, inversion, chromothripsis[23], and read-through[24]. The first identified cancer-causing fusion gene is BCR-ABL gene, product of a reciprocal interchromosomal translocation between the q arms of chromosomes 9 and 22 that occurs in more than 96% of patients with CML[25]. The first fusion gene in epithelial solid tumors, rearranged during transfection (RET)-CCDC6, was found in papillary thyroid carcinoma more than 30 years ago[26]. Since then, many gene fusions have been discovered, facilitated by large scale sequencing efforts such as those championed by The Cancer Genome Atlas (TCGA), International Collaboration for Clinical Genomics (ICCG), International Cancer Genome Consortium (ICGC), and numerous other institutional studies. With the technological advancement in detection methods, the identity of gene rearrangement partners, the spectrum of tumor histologies where the gene rearrangements have been found, and their overall prevalence have significantly expanded in the past few years.

For instance, a recent study by Gao *et al.*[27] interrogated 9,624 samples belonging to 33 cancer types in the TCGA collection and identified 25,664 distinct fusion events. Importantly, among all fusions involving receptor and non-receptor kinases, 1,275 cases contain an intact kinase domain, many of which are believed to be the sole onco-driver in a particular tumor biopsy. Many of these fusion events lead to constitutive activation of the kinase activity and downstream signaling pathways including mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) cascades, which enables cells to hyper-proliferate and evade apoptosis[28-30] [Figure 2]. The mechanisms of activation include overexpression of the kinase as a result of the activity of the promoter of the fusion partner, constitutive ligand-independent dimerization of the fusion kinase proteins, and release of kinase auto-inhibitory mechanism. Since kinases are generally druggable targets, studies such as this provided the rationale for developing small molecule targeted therapies to treat fusion-driven hematological and solid tumors[31-34].

THE LONG-TAIL PHENOMENON AND TISSUE-AGNOSTIC DEVELOPMENT

Although conceivably, specific drugs can be developed to address these distinct fusion proteins in each of the tumor types involved individually, in reality, with the exception of a few cases, such as ALK and ROS1 fusions in NSCLC[35] and fibroblast growth factor receptor (FGFR) fusions in cholangiocarcinoma[36], the majority of the fusions occur at low frequencies[37]. The low and ultra-low frequency alterations sometimes are called the "long tail"[38]. As discussed above, the rarity of the fusions and the resulting small patient pool make the development of a particular targeted drug for a single tumor type impractical.

One potential solution to address this challenge lies in the observation that a number of recurring gene fusions, such as those formed by ALK, ROS1, FGFR, NTRK, and RET, have been identified in multiple cancer histologies. For example, ALK fusions are found in anaplastic large cell lymphoma[39], NSCLC[40], papillary thyroid cancer[41], colorectal cancer[42], renal cell cancer[43], and esophageal cancer[44], as well as in spitzoid tumors[45]. Similar to ALK fusions, FGFR fusions have been reported in a wide range of tumors such as cholangiocarcinoma, breast cancer, prostate cancer, NSCLC, gastric adenocarcinoma, colorectal adenocarcinoma, and glioblastoma, with a large number of distinct fusion partners[46]. The long-tail phenomena (rare and ultra-rare patient populations) and recurring fusions across multiple tumor types necessitate biomarker-driven cross-tumor type clinical trials, to enroll a sufficient number of patients for efficacy and safety assessment and to offer patients with a rare actionable mutation access to an experimental therapy.

TRK

The tyrosine kinase receptors TRKA, TRKB, and TRKC, are encoded by neurotrophic tropomyosin receptor kinase (*NTRK*) genes *NTRK1*, *NTRK2*, and *NTRK3*, respectively. Their ligands are neurotrophins, a family

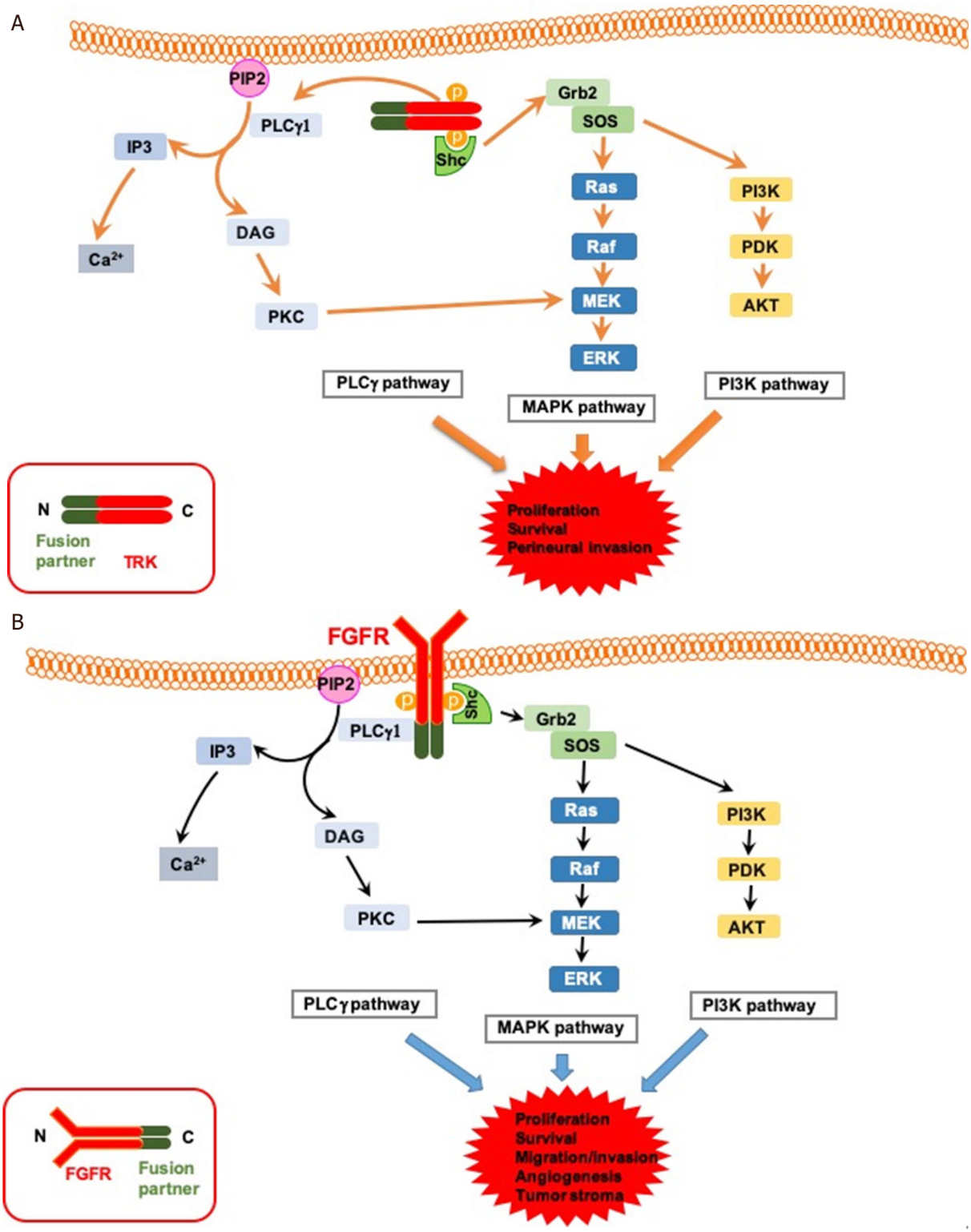

Figure 2. Oncogenic fusions lead to ligand-independent, constitutive activation of kinases and their downstream signal pathways. A: Fusions with *N*-terminal partners, represented by tropomyosin receptor kinase (TRK) fusions; B: fusions with *C*-terminal partners, represented by fibroblast growth factor receptor fusions. AKT: protein kinase B

of nerve growth factors including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins[47]. Neurotrophin binding to TRK receptors results in receptor autophosphorylation

and activation of downstream signaling cascades. Physiologically, the TRK family members play a key role in normal central and peripheral neuronal cell development and differentiation. *NTRK* gene mutations, overexpression, splice variants, and fusions/rearrangements have been found in a number of human cancer types[48].

The first *NTRK* gene fusion, *TPM3-NTRK1*, was identified in a colon carcinoma biopsy through a transformation assay[49]. Subsequently, *TPM3-NTRK1* has been identified in additional CRC tumors[50], sarcoma[51], lipofibromatosis-like neural tumors[52], spitzoid melanoma[53], invasive mucinous adenocarcinoma of the lung[54], and papillary thyroid carcinoma[55]. With the use of advanced molecular diagnostic techniques, additional *NTRK1* fusion partners have been identified in various tumor types, with 5' fusion partners including LMNA, SQSTM1, BCAN, CD74, IRF2BP2, MDM4, MPRIP, and others[56].

Similarly, oncogenic fusions have also been identified with *NTRK2* and *NTRK3* genes, as well as across a variety of cancer histologies. For example, *NTRK2* forms fusions with partners such as *AGBL4*, *NACC2*, *QKI*, and *VCL*, which were identified in non-brainstem high-grade glioma, soft tissue liposarcoma, head and neck squamous cell carcinoma, pilocytic astrocytoma, ganglioglioma, and diffuse intrinsic pontine glioma. *NTRK3* fusion partners include *ETV6*, *BTBD1*, and *EML4*, which were identified in diffuse intrinsic pontine glioma, congenital fibrosarcoma, papillary thyroid carcinoma, mammary analog secretory carcinoma (MASC) of the thyroid gland, secretory breast cancer, and inflammatory myofibroblastic tumor[48,57-60], as well as in hematological malignancies such as acute myeloid leukemia, Philadelphia-like acute lymphoblastic leukemia, and chronic eosinophilic leukemia[61-63].

NTRK gene fusions have also been reported in pediatric solid tumors. For instance, oncogenic gene fusions involving the *NTRK3* kinase domain have been identified in congenital fibrosarcoma and pediatric mesoblastic nephroma and acute leukemias[57]. A survey of 2 pediatric cancer databases, St. Jude pediatric cancer database (PeCan; total n = 1,604) and the University of Michigan database (Peds-MiOncoSeq; total n = 91) resulted in the identification of three gene rearranged-cancers, one each involving *NTRK1*, *NTRK2*, and *NTRK3* in a sarcoma, a low-grade glioma, and a B-cell acute lymphoblastic leukemia, respectively. In addition, the following tumor types, which are largely confined to the pediatric patient population, are also known to harbor *NTRK* gene fusions: congenital or infantile fibrosarcoma, secretory (juvenile) breast cancer, mesoblastic nephroma, and intrinsic pontine gliomas[64].

Fusion of 3' *NTRK* gene sequences encoding the tyrosine kinase domain to various 5' partner sequences via intra- or inter-chromosomal rearrangement results in an oncogenic chimera protein that can ligand-independently homodimerize, autophosphorylate, and constitutively activate downstream signaling pathways, such as MAPK, PI3K/protein kinase B (AKT), and phospholipase C (PLC)-γ, which can result in hyperproliferation and cell survival in tumors expressing these proteins. The growth of cancer cells thus becomes dependent on or "addicted" to this abnormal kinase signaling[65].

Although oncogenic *NTRK* gene fusions are observed across a large number of adult and pediatric solid and hematological tumors, they are rare events in most common cancers (e.g., frequency of < 0.1% in NSCLC or CRC). Although much higher frequencies of *NTRK* fusions are present in certain tumor types such as MASC, these cancers are ultra-rare, collectively representing less than 1% of all malignancies. As a result, the overall population of *NTRK*-fusion-positive patients is very small[66].

The rarity of the molecularly defined patients and the vastly diverse histologies of the patients clearly called for an innovative, tissue-agnostic approach. Fortunately, data generated in preclinical studies provided rationale to perform tissue-agnostic clinical trials in multiple molecularly defined cancers[56]: (1) regardless of the fusion partner or the tissue of origin, the *NTRK* gene fusions result in a constitutively active kinase

and provide the driving force for transformation and tumor progression; and (2) regardless of the identity of the fusion partners, TRK inhibitors such as entrectinib exhibit similar anti-tumor potency in cell lines harboring *NTRK1*, *NTRK2*, or *NTRK3* fusion genes (i.e., *TPM3-NTRK1*, *LMNA-NTRK1*, *SQSTM1-NTRK1*, *BCAN-NTRK1*, *MPRIP-NTRK1*, *AFAP1-NTRK2*, *VCL-NTRK2*, and *ETV6-NTRK3*), and in *NTRK*-fusion-positive xenograft models derived from various tumor types. For instance, tumor growth inhibition was observed in cancer cell line-derived xenograft models of CRC harboring *TPM3-NTRK1* fusion, AML harboring *ETV6-NTRK3* fusion, and NSCLC harboring MPRIP-*NTRK1* fusion, as well as in patient-derived xenograft (PDX) models of metastatic CRC harboring *LMNA-NTRK1* fusion, head and neck cancer harboring *ETV6-NTRK3* fusion, and sarcoma harboring *TPM3-NTRK1* fusion[67].

As discussed above, the preclinical observations have been clinically validated in several clinical trials that led to the regulatory approvals of larotrectinib and entrectinib as the first two small molecule anti-cancer drugs that carry a tissue-agnostic label.

ROS1

ROS1 belongs to the insulin-receptor superfamily of receptor tyrosine kinases and plays a role in relaying growth signals from the environment outside the cell into the cell's nucleus. It is an orphan receptor tyrosine kinase with no known binding ligand. Genetic changes in *ROS1*, such as gene rearrangements, mutations, or copy number increases, create oncogenes that can lead to cancer[68]. *ROS1* gene rearrangements create fusion proteins with constitutively active kinase domains that activate downstream signaling pathways leading to oncogenic properties in cells, including uncontrolled proliferation and resistance to cell death with prolonged tumor cell survival. These pathways include Ras-ERK for cellular proliferation and the Janus kinase/signal transducer and activator of transcription (JAK/STAT) and PI3K/AKT pathways, which regulate cell survival (anti-apoptosis) and proliferation. *ROS1* fusion proteins may also activate the mammalian target of the rapamycin pathway, which is critical for the regulation of protein translation. Cancers that have these pathways activated tend to be more aggressive, with invasion and metastasis leading to poor patient survival[69].

In NSCLC patients, *ROS1* fusion protein is found in approximately 1%-2.5% of patients[70,71]. *ROS1* gene rearrangements have also been detected in a variety of other cancers, including glioblastoma multiforme[72,73]; biliary tract carcinoma (3.9%)[74]; ovarian cancer, gastric adenocarcinoma (0.61%)[75]; CRC (0.85%)[76]; inflammatory myofibroblastic tumor, angiosarcoma, and epithelioid hemangioendothelioma[69,70,75,77]; and Spitz nevus (benign) (25.3%), atypical Spitz tumors (6.2%), and spitzoid melanomas (9.1%)[45].

Thus far, more than two dozen *N*-terminal fusion partners have been identified[78]. All the fusion proteins retain the *ROS1* kinase domain, but rarely its transmembrane domain[79]. The most common *ROS1* fusion partner is CD74[80]. Other commonly observed *ROS1* fusion partners include *SDC4*, *SLC34A2*, *LRIG3*, *EZR*, and *TPM3*[77,78]. A survey of cBioPortal for Cancer Genomics (https://www.cbioportal.org) and The Cancer Genome Atlas (TCGA) generated the following breakdown of *ROS1* fusion partners: 38% *CD74*, 12% *EZR*, 12% *SLC34A2*, 9% *SDC4*, 6% *CEP85L*, 6% *GOPC*, and rare cases of *CLTC*, *GOLGB1*, *SLC4A4*, *TFG*, *TMEM181*, and *TPM3*. More than half of the partners have dimerization domains that are retained in the fusion, presumably leading to constitutive *ROS1* tyrosine kinase activation. Additional mechanism of activation of the *ROS1* fusion proteins may include removal of the auto-inhibitory domain from the full-length *ROS1* as the result of the fusing event[69]. Recent survey of responses to crizotinib in 106 NSCLC patients with *ROS1* fusions of various fusion partners (49.1% *CD74*, 17% *EZR*, 14.2% *SDC4* and 4.7% *TPM3*) showed no significant difference among patients with various types of *ROS1* fusion partners in overall survival (OS) and progression-free survival (PFS)[81].

Clinically, multiple ROS1 inhibitors have been approved for *ROS1*-fusion-positive NSCLC. Although the clinical efficacy of *ROS1* inhibitors has not been systemically established, several preclinical studies have

shown support of potential broad efficacy across tumor types that are driven by *ROS* fusion. For instance, Davare *et al.*[73] showed that *CEP85L-ROS1* and *GOPC-ROS1* are transforming oncogenes in cells of astrocytic lineage, and they are sensitive to pharmacologic inhibition with several *ROS1* inhibitors *in vitro*. Furthermore, systemic therapy with a BBB-penetrant *ROS1* inhibitor, lorlatinib, significantly prolonged survival in an intracranially xenografted, *ROS1*-fusion-positive glioblastoma tumor model. In a separate study[82], *ROS1* inhibitors were able to inhibit FIG-ROS-driven cholangiocarcinoma *in vitro* and *in vivo*. These data provide the rational support to a potential tissue-agnostic approach for treating ROS1-fusion-positive cancers.

ALK

ALK belongs to the insulin-receptor superfamily and aberrant ALK fusion proteins lead to self-activation and constitutive activity within cancer cells via activation of signal transduction pathways and intracellular kinases that drive uncontrolled tumor cell growth, metabolism, and survival[83]. In addition to anaplastic lymphomas (ALCL), ALK oncogenes are found in a number of cancers such as NSCLC, diffuse large B-cell lymphoma, neuroblastomas, colorectal cancer, inflammatory myofibroblastic tumors (IMT), esophageal/gastric cancers, and renal cell cancers[35,83]. The currently available ALK inhibitor drugs, crizotinib, ceritinib, alectinib, and brigatinib, have demonstrated clinical benefit in NSCLC[84]. In a recent report based on the CREATE Study in eight European countries, Schoffski *et al.*[85] showed that, in ALK-fusion-positive IMT, crizotinib treatment resulted in 50% ORR (6 responders out of 12 patients) with nine-month median duration of response and 73% one-year PFS. On the contrary, the ORR was 14% (one out of seven) in ALK-negative IMT. In addition to the CREATE study, similar or higher ORRs were observed in ALK-fusion-positive patients enrolled in studies COG and PROFILE 1013. These two studies also demonstrated ORRs of 53%-88% in ALCL.

FGFR

The FGFR protein family consists of four highly conserved transmembrane receptor tyrosine kinases (FGFR1-4). Receptor activation by the fibroblast growth factor (FGF) ligands or oncogenic alterations leads to intracellular signaling to promote cell proliferation, differentiation, morphogenesis and patterning, angiogenesis, and survival[86]. The FGFR signaling pathway is aberrantly activated in multiple types of human cancers through various molecular alterations including point mutations, gene amplification and overexpression, and chromosomal rearrangements/translocations. Many of these changes lead to constitutive receptor activation and upregulation of the downstream signaling pathways, leading to uncontrolled cell proliferation, survival, and migration, which are hallmarks of cancer. Both the overall frequency of FGFR alterations and the relative distribution of the types of alterations vary by cancer type[46,87,88].

FGFR fusions are the result of gene rearrangements and have been detected in different types of human cancers[46,89] [Table 1]. Particularly, *FGFR2* fusions with different partners, such as *BICC1*, *TACC3*, *CCDC6*, and *AHCYL1*, have been detected in approximately 10%-20% of intrahepatic cholangiocarcinomas[90-92]. Lower frequencies of *FGFR1-3* fusions have also been detected in breast cancer, bladder cancer, glioblastoma, head and neck squamous cell carcinoma, low-grade glioma, lung adenocarcinoma, lung squamous cell carcinoma, ovarian cancer, prostate adenocarcinoma, and thyroid carcinoma[46,87,88,93]. *FGFR* fusion partners generally contain dimerization or oligomerization domains that lead to ligand-independent constitute activation of the receptor and downstream RAS-MAPK and JAK-STAT signaling pathways[88], resulting in uncontrolled cell proliferation, survival, and migration, which are hallmarks of cancer. In solid tumors, these *FGFR* fusions are typically formed by fusing near full-length *FGFR* with intact kinase domain at the *N*-terminus and various fusion partners at the *C*-terminus (Type II fusions), suggesting that these may serve as potential therapeutic targets via kinase inhibition. However, it is also possible for the fusion to

Table 1. FGFR fusions and fusion partners in solid tumors

Cancer Type	5′-gene	3′-gene
Bladder	*FGFR1*	*NTM*
	FGFR3	*TACC3, TNIP2, JAKMIP1, BAIAP2L1*
Breast	*FGFR1*	*ADAM18*
	RHOT1	*FGFR1*
	NSD3	*FGFR1*
	ERLIN2	*FGFR1*
	FGFR2	*CCDC6, AFF3, CASP7, NCALD, WHSC1*
Cervical cancer	*FGFR3*	*TACC3*
Cholangiocarcinoma	*FGFR2*	*AFF4, AHCYL1, BICC1, CCDC6, VCL, CLIP1, POC1B, CELF2, CREB5, DNAJC12, HOOK1, KCTD1, KIAA1217, KIAA1598, MGEA5, NOL4, OPTN, PARK2, PCMI, PPHLN1, RASAL2, SLMAP2, SORBS1, STK26, STK3, TACC3, TBC1D1, TFEC, TRA2B, UBQLN1, WAC, ZMYM4*
Colorectal cancer	*FGFR2*	*NPM1, COL14A1*
Gastric cancer	*FGFR2*	*C100rf68, PDHX, TACC2*
Glioblastoma	*FGFR3*	*TACC3*
Head and neck squamous cell carcinoma	*FGFR3*	*TACC3, TPRG1*
Lung squamous cell carcinoma	*BAG4*	*FGFR1*
	FGFR2	*CCAR2, CIT, KIAA1967*
	CCAR2	*FGFR2*
	FGFR3	*TACC3*
Mesothelioma	*FGFR2*	*CASC15*
Ovarian cancer	*FGFR2*	*USP10*
Prostate adenocarcinoma	*FGFR2*	*KLK2, PPAPDC1A, SLC45A3*
	FGFR3	*AES*
Renal cell carcinoma	*FGFR3*	*TACC3*
Thyroid cancer	*FGFR2*	*OFD1*
	VCL	*FGFR2*

FGFR: Fibroblast growth factor receptor

occur so that the *FGFR* gene remains intact on the 3' end of the gene (Type I fusions) allowing the fusion partner to be present on the 5' end[88,94]; these fusions are mostly found in hematological malignancies[95].

Given the significance of constitutive *FGFR* signaling in tumorigenesis and progression, small molecule inhibitors targeting this pathway have been developed and their anti-tumor activities are currently being evaluated in clinical trials[88,96,97]. For instance, recent results from several clinical trials in *FGFR2*-fusion-positive cholangiocarcinoma have demonstrated meaningful clinical efficacy, which supports potential approvals as second line therapy for the treatment of advanced cholangiocarcinoma with *FGFR2* fusions. For example, in a Phase II study of infigratinib in 71 cholangiocarcinoma patients with *FGFR2* fusions, ORR 31% and SD 58% were observed, with median PFS and OS of 6.8 and 12.5 months, respectively[98]. Similar results were reported based on an interim update from the Phase II study of pemigatinib in *FGFR2*-fusion-positive cholangiocarcinoma patients[99]. Erdatinib, recently approved for advanced or metastatic urothelial carcinoma with susceptible *FGFR3* mutations, has demonstrated efficacy in therapeutic trials for cholangiocarcinoma patients with *FGFR2* fusions, although these trials contained fewer patients[100]. Additionally, a covalent pan-*FGFR* inhibitor, futibatinib, has shown limited efficacy in cholangiocarcinoma patients previously treated with a different *FGFR* inhibitor, suggesting a potential utility for later line therapy when drug sequencing is needed[101].

In addition to cholangiocarcinoma, there is evidence, albeit very limited, that *FGFR* inhibitors work in other solid tumors with *FGFR1*, -2, or -3 fusions[97,102-104]. Based on these data there is a rationale for performing tumor-agnostic clinical trials in molecularly defined cancers to maximally benefit patients with serious and life-threatening diseases.

RET

RET encodes a single-pass transmembrane receptor tyrosine kinase important for normal cellular proliferation, development, and maintenance. It has four cadherin-like repeats at the *N*-terminal

extracellular domain, a cysteine-rich region, a transmembrane domain, and *C*-terminal cytoplasmic tyrosine kinase domain[105]. Under normal conditions, wild-type *RET* is activated through binding of glial cell line-derived neurotrophic factor (GDNF) family of ligands[106] and a co-receptor, and it functions through the modulation of downstream signaling including RAS-MAPK, PI3K-AKT, and phospholipase Cγ (PLCγ) pathways[107,108]. During development, RET protein plays an important role in the development of the enteric nervous system[109] and homeostasis of neural, neuroendocrine, hematopoietic, and male germ tissues[106,110].

In certain cancers, aberrant, ligand-independent *RET* activation is associated with gain of function *RET* mutations or gene rearrangements (fusions). The first known *RET* fusion was an in-frame fusion of *CCDC6-RET* identified in a patient with papillary thyroid carcinoma[111]. Subsequently, *RET* fusions were reported in 13%-43% of papillary thyroid carcinomas[112], and multiple fusion partners have been reported. *RET* fusions mostly occur in irradiation-induced papillary thyroid carcinoma[112].

The first group of *RET* fusions in lung cancer, *KIF5B-RET*, was reported in 2012[113] with estimated incidence rates between 1.3% and 6% of lung adenocarcinomas tested. The *KIF5B-RET* fusion contains *RET* kinase domain fused with a coiled-coil domain from *KIF5B*, which mediates homodimerization and ligand-independently activates the oncogenic pathways by autophosphorylation. It is also believed that the fusion event eliminates the auto-inhibitory domain of *RET*[107,108]. Later studies with much larger samples put the fusion rate at 1%-2%[114,115]. Interestingly, a review of 936 patients with surgically resected NSCLC suggested that *RET*-fusion-positive patients tended to be associated with younger age, never-smoker status, early lymph node metastases, poor differentiation, and a solid-predominant subtype[116]. Not surprisingly, and similar to other oncogenic drivers, *RET* fusions are largely mutually exclusive from other known oncogenic alterations[117].

Besides coiled-coil domain-containing protein 6 (*CCDC6*) and *KIF5B*, other partners of *RET* include nuclear receptor coactivator 4 (*NCOA4*), the tripartite motif-containing 33 (*TRIM33*), myosin VC gene (*MYO5C*), EPH receptor A5 gene (*EPHA5*), CAP-Gly domain containing linker protein family member one gene (*CLIP1*), ELKS/RAB6-interacting/CAST family member one gene (*ERC1*), phosphatidylinositol binding clathrin assembly protein gene (*PICALM*), FERM domain containing 4A gene (*FRMD4A*) *RUN*, *RYVE* domain containing two gene (*RUFY2*), tripartite motif containing 24 gene (*TRIM24*), tripartite motif containing 27, and many others. All of these fusion counterparts have a dimerization domain that induces ligand-independent activation of the *RET* kinase[118-120].

In addition to papillary thyroid cancer and lung adenocarcinoma, *RET* fusions have been identified in other solid tumors, including colorectal cancer (CRC)[121,122], breast cancer[121], and Spitz tumor[45,123] [Table 2].

Given the importance of *RET* fusions in cancer biology and preclinical support of targeting *RET* as a potential intervention agent for cancer[124,125], multiple selective small molecule kinase inhibitors, such as *RXDX-105*[124], *BLU-667*[126], and *LOXO-292*[127], have entered clinical development. In the most recent update[128], *LOXO-292* demonstrated a 68% ORR in *RET*-fusion-positive NSCLC patients who had previously received chemotherapy. Additionally, *BLU-667*[129] achieved 60% response in second line *RET*-fusion-positive NSCLC and 63% response in *RET*-altered medullary thyroid cancer (MTC) who had previously been treated with Caprelsa or Cabometyx.

CHALLENGES

Six years ago, Lacombe *et al.*[130] in their opinion article "The dream and reality of histology agnostic cancer clinical trials", while appreciating the need and advantages of tissue-agnostic trials, expressed great uncertainty whether a true tissue-agnostic approach was feasible and approvable. Six years later, however,

Table 2. RET fusions and fusion partners in solid tumors other than lung cancer

Tumor histology	RET fusion detection rate (%)	Fusion partners
PTC	6% (Kondo *et al*.[112] 2006, Stransky *et al*.[121] 2014)	*AKAP13, FKBP15, HOOK3, PCM1, PRKAR1A, SPECC1L, TBL1XR1, TRIM24, TRIM27, CCDC6, ERC1, KIF5B, NCOA4, GOLGA5, KTN1, RFG9*
CRC	0.2%-0.4% (Stransky *et al*.[121] 2014, Le Rolle *et al*.[122] 2015)	*CCDC6, NCOA4*
BC	0.1% (Stransky *et al*.[121] 2014)	*ERC1*
Spitz tumors	3% (Wiesner *et al*.[45] 2014)	*GOLGA5, KIF5B*

PTC: Papillary thyroid cancer; CRC: colorectal cancer; BC: breast cancer; RET: rearranged during transfection

with three tissue-agnostic cancer drugs approved and more than a dozen in various stages of development, the tissue-agnostic approach is becoming a viable route for demonstrating efficacy of a targeted agent in multitude of tumor types with shared molecular aberration or target as the common denominator. This approach is especially attractive for those cancers with rare or ultra-rare patient populations. At the same time, it is important to acknowledge that there are still many challenges and limitations in this emerging area of research and development.

The first challenge is to determine, at the target and biology levels, whether same aberrations in different histologies have similar biological, functional, and pathological significance. The preclinical data and clinical experience in targeting *NTRK* fusions clearly confirmed that *NTRK* fusions are the single dominant oncogenic driver in fusion positive cancers, independent of tissue origin of the cancer[131]. Therefore, *NTRK* fusions represent an ideal tissue-agnostic target. On the other hand, one of the prominent failures during early days of tissue-agnostic exploration involved BRAF targeting in different tumors including melanoma, thyroid carcinoma, and colorectal cancer[132]. Whereas vemurafenib was efficacious in BRAF V600E melanoma[133] and thyroid carcinomas[134], it failed to halt colorectal cancer with the same BRAF mutation[135], partly due to a tissue-specific feedback activation of EGFR pathway in CRC patients[136]. This exemplifies the role of histological context plays in certain cancers that influence the drug-target response. It is unclear what level of influence the tissue context has on the oncogenic fusions. Will the oncogenic fusions of *ALK*, *ROS1*, *FGFR*, and *RET* behave similarly to *NTRK* fusions upon treatment? For example, it has been shown that different *ROS1* fusions exhibited different subcellular localizations[79], which could lead to varied levels of activation and pathway involvement. Whether differential subcellular localization is a more general feature regulating oncogenesis across different oncoprotein fusions remains unclear. Therefore, extensive translational research efforts need to be an integral part of these trials to guide patient selection strategy.

The clinical development path for tissue-agnostic indication can be challenging. For instance, how is the sample size determined for each of the tumor types? What are the common endpoints, considering each tumor type is likely to have distinct natural history, standard of care option(s) and treatment algorithm (line of therapy), reference response rates and duration of response, and survival end points? Particularly, response assessment criteria would require cross-tumor harmonization, since these can differ depending on tumor type. There is no standard design of basket trials, especially for the very rare and ultra-rare patient populations. For instance, larotrectinib was conditionally approved based on a 55-patient trial that spanned 12 distinct tumor types, some of which were represented by just one patient. Will this happen to a future trial and still get approved? It is obvious that in these trials the statistical analyses are different from well-established practices and innovative approach will be needed to support drug development decisions. Operationally, basket trials require well-coordinated effort from different specialists and their teams of the respective departments, which are typically organized by organ site. This holds particularly true for the collection and processing of the patients' biological material for molecular diagnostics.

The challenges in the regulatory processes should not be ignored either. Regulatory agencies in different countries and geographic regions, such as US/North America, European Union, Japan, and other Asia-Pacific countries, may have different degrees of acceptance of the tissue-agnostic approach. It is encouraging that recently the regulatory authorities in the US (larotrectinib and entrectinib), EU (larotrectinib), and Japan (entrectinib) all gave the green light to tissue-agnostic indication for *NTRK* fusions. However, the comfort level of these agencies may be different if the next agent does not have the splendid response rates larotrectinib and entrectinib exhibited, and the future approvals are likely to be reviewed on case-by-case basis and no established playbook exists.

CONCLUSION

With the rapid advances in cancer genomics, drug design and precision diagnostics, the field of oncology drug development has entered an era when both traditional tissue-restricted and innovative tissue-agnostic approaches provide precedented approval paths. Despite the challenges, we anticipate that tissue-agnostic approvals will continue to grow and expand the therapeutic options for cancer patients in need.

DECLARATIONS

Authors' contributions

Researched and gathered the material and data: Li IW
Reviewed the manuscript: Li IW, Wei G, Li G
Drafted the manuscript: Krishnamurthy N, Wei G, Li G
Proposed the concept: Li G

Availability of data and materials

Not applicable.

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Conflicts of interest

Wei G and Li G are employees of QED Therapeutics and equity owners.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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