IN THE SPOTLIGHT

The Democratization of the Oncogene

Anh T. Le and Robert C. Doebele

Summary: The identification of novel, oncogenic gene rearrangements in inflammatory myofibroblastic tumor demonstrates the potential of next-generation sequencing (NGS) platforms for the detection of therapeutically relevant oncogenes across multiple tumor types, but raises significant questions relating to the investigation of targeted therapies in this new era of widespread NGS testing. *Cancer Discov; 4(8); 870–2. ©2014 AACR.*

See related article by Lovly et al., p. 889 (4).

Historically, malignancies have been classified, staged, and treated on the basis of histologic criteria (e.g., adenocarcinoma vs. squamous cell histology) and the organ site of origin (e.g., lung vs. colon). This classification system is useful because these diseases often demonstrate similar features such as symptoms, patterns of metastatic spread, and prognosis, and therefore allows the study of new treatments in a systematic fashion in a relatively uniform group of patients. The initial detection of therapeutically relevant oncogenic alterations, such as *HER2* (gene amplification) in breast cancer, *BCR-ABL* (gene fusion) in chronic myelogenous leukemia, and *EGFR* (activating mutations) in lung cancer, did little to disrupt this paradigm, as each of these oncogenes was primarily restricted to one disease type.

ROS1 fusions were first fully characterized in a glioblastoma cell line in 2003 and later identified in a non–small cell lung cancer (NSCLC) cell line in 2007 (1, 2). Since then, *ROS1* gene fusions have been identified in approximately 1% of patients with lung cancer and, until now, all of the clinical data relating to effectiveness of ROS1 inhibitors has been performed in patients with lung cancer (3). Although this seems to be a small percentage, this accounts for approximately 2,000 patients per year in the United States. Clinical trials of rare genotypes in NSCLC are enabled by the large patient population and the existing routine testing of other actionable oncogenes (*ALK* and *EGFR*) in this disease, which facilitates the testing of additional oncogenes in this tumor.

Similar to *ALK* or other gene fusions, *ROS1* fusions contain sequences from a 5′ partner gene fused in-frame to the 3′ portion of the *ROS1* gene, which encodes the kinase domain. Expression of the *ROS1* fusions by the promoter of the 5′ partner and replacement of 5′ *ROS1* sequences encoding the extracellular domain of *ROS1* lead to constitutive activation of the ROS1 kinase. Numerous 5′ partner genes have been implicated in *ROS1* rearrangements (Fig. 1A). *ROS1* is highly homologous to *ALK* and is inhibited by crizotinib, an FDA-approved therapy for anaplastic lymphoma kinase positive (ALK^+) NSCLC (3).

The study by Lovly and colleagues (4) in this issue identified *ROS1* fusions in a significant portion of patients with inflammatory myofibroblastic tumors (IMT). Thirty-seven IMTs underwent genomic DNA sequencing using a commercially available targeted next-generation sequencing (NGS) assay (FoundationOne). Four of 37 samples (~11%) demonstrated evidence of an oncogenic *ROS1* fusion. Two novel *ROS1* fusions were identified in this study, *YWHAE–ROS1* and *TFG–ROS1*, expanding further the diversity of genes known to rearrange with *ROS1* (Fig. 1A). This was the first identification of *ROS1* fusions in this cancer type, expanding the number of tumor types already known to harbor this oncogene: NSCLC, colon cancer, gastric cancer, cholangiocarcinoma, angiosarcoma, glioblastoma, Spitzoid neoplasms, and ovarian cancer (Fig. 1B; refs. 3, 5). Importantly, the authors describe the successful treatment of a ROS1-positive IMT patient with crizotinib, the first report of a non–lung cancer ROS1-positive patient treated successfully with a ROS1-specific kinase inhibitor. The study was also the first to identify *PDGFRB* gene fusions in IMTs, a class of oncogene previously described in myeloid neoplasms associated with eosinophilia and with demonstrated responses to imatinib or other kinase inhibitors (6). *ALK* fusions were reidentified in a large portion of IMTs in this study; responses to the ALK inhibitor crizotinib have previously been described (7). In sum, all but 3 of 37 samples had evidence of an oncogenic fusion involving *ROS1*, *PDGFRB*, or *ALK*, suggesting that gene rearrangements are the predominant, if not the sole, driver in this tumor type and that most IMTs should be susceptible to a targeted therapy.

This study therefore highlights several important questions in the era of widely available NGS. First, if a new mutation/alteration is found in a known oncogene (e.g., a new gene rearranged with *ROS1*), should it be presumed susceptible to a targeted therapy that has already demonstrated success for that class of oncogene? A mutation in a proto-oncogene does not always confer oncogenicity; for example, a single-nucleotide polymorphism with a known germline prevalence or a mutation conferring a conservative amino acid substitution that has little or no effect on protein structure or function is unlikely to be

Authors' Affiliation: Department of Medicine, Division of Medical Oncology, University of Colorado School of Medicine, Aurora, Colorado

Corresponding Author: Robert C. Doebele, Department of Medicine, Division of Medical Oncology, University of Colorado School of Medicine, 12801 E. 17th Avenue, Aurora, CO 80045. Phone: 303-724-2980; Fax: 303-724-3889; E-mail: robert.doebele@ucdenver.edu

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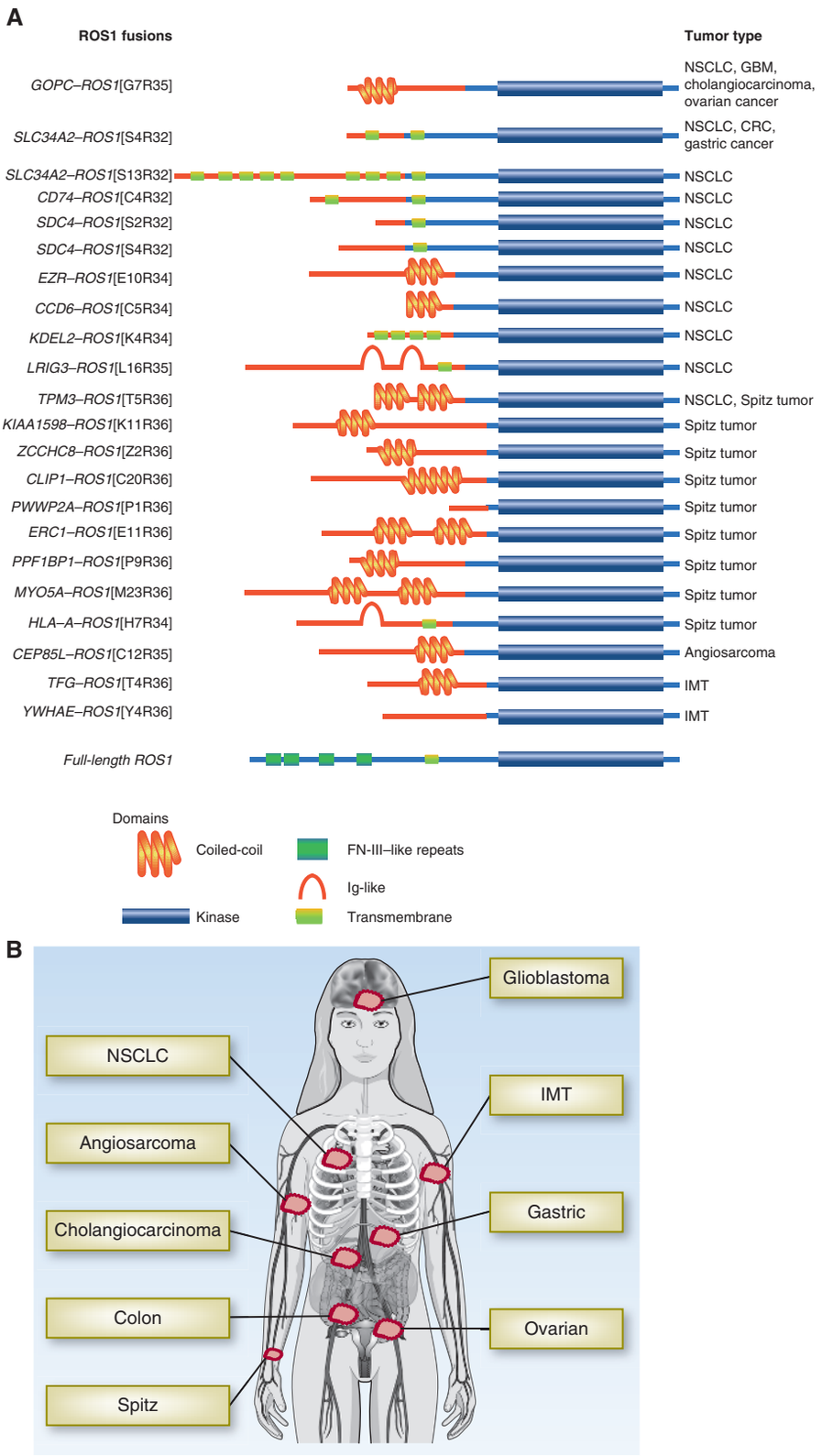

Figure 1. A variety of *ROS1* gene fusions occur across multiple tumor types. **A,** schematic of oncogenic *ROS1* fusions identified to date, illustrating 20 different 5′ gene fusion partners that rearrange with *ROS1* in cancer. Exon variants are shown in brackets with gene names abbreviated to first letter. **B,** illustration of tumor types identified thus far that can harbor *ROS1* gene fusions. GBM, glioblastoma multiforme; CRC, colorectal cancer.

oncogenic and therefore also unlikely to be clinically significant. In the case of gene fusions, multiple different rearrangements can confer oncogenicity; the minimal necessary requirements are that the rearrangement generates an in-frame transcript and that this transcript encodes an intact kinase domain (3, 6). This study identified several new fusions involving *ALK*, *ROS1*, and *PDGFRB*. All generate an in-frame fusion leaving the respective kinase domains intact and are therefore likely to be oncogenic by meeting these basic criteria of fusion genes; however, it remains a possibility that fusion genes identified by NGS or other tests will not always be functional or will not respond to targeted therapy based on the cellular or genetic context.

Second, if an oncogene is susceptible to targeted therapy in one disease (e.g., *ROS1* fusions in NSCLC), is that sufficient evidence to treat patients with a similar oncogene in another tumor type? Thus far, there are examples to argue for and against the argument that an oncogene will respond similarly to targeted therapies in different tumor-type contexts (4, 7, 8)—only continued testing of this hypothesis will determine where the preponderance of evidence lies. As NGS testing becomes more widespread, this scenario is only likely to become more common. Ideally, patients with a characterized oncogene, but in a new tumor type, would be enrolled on clinical trials for formal and rigorous hypothesis testing (e.g., ALK-positive IMTs enrolled on the phase I expansion trial of crizotinib; NCT00585195; ref. 7). There are several existing barriers to this desired approach, however, including the potential rarity of an oncogene in a tumor type (e.g., *ROS1* fusions in colorectal cancer or ovarian cancer; refs. 9, 10), the rarity of the tumor itself (e.g., IMTs), lack of geographical access to a clinical trial site, and/or the widespread and growing availability of multiple FDA-approved oral kinase inhibitors that cover an increasing number of oncogene targets, potentially facilitating the use of off-label therapies. All of these factors will make it difficult, but not impossible, to formally study infrequent oncogenes in each tumor type, despite the increasing ease of identifying these oncogenes. Although oncogenes can occur at low frequencies in a given tumor type, it seems imperative to bring the potential of targeted therapy to any patient with an actionable oncogene based on the dramatic responses and prolonged progression-free survival often observed for targeted therapies in oncogene-driven cancers. One might propose large, NGS-driven trials across multiple tumor types (a so-called "master protocol" approach), but choosing the markers and drugs to be studied could pose a significant logistical challenge. The current organ-based clinic structure of most academic medical centers is an obstacle to enrolling patients with different tumor types in a single clinical trial addressing one class of oncogene ("basket approach"), with phase I clinical trial programs that accommodate multiple tumor types being the exception, but perhaps not perfectly suited to phase II/III trials when the dose and safety of a drug are well-established. Finally, the proliferation of NGS testing may encourage the establishment of "Molecular Tumor Boards" to bring together medical oncologists from different subspecialties, pathologists, and basic/translational scientists to facilitate discussions around oncogene testing and decision-making for clinical trial enrollment and/or treatment decisions. In conclusion, current advancements in the detection of oncogenes by NGS or other methods will force us to rethink our current infrastructure for testing new therapies in patients with cancer.

Disclosure of Potential Conflicts of Interest

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