SIMPLIFYING YOUR APPROACH TO PRECISION ONCOLOGY

Understanding Pathogenic Gene Fusions and the Role of RNA-Based Genomic Testing

Merus closing in on cancer

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NGS, next-generation sequencing; *NRG1*, neuregulin 1.

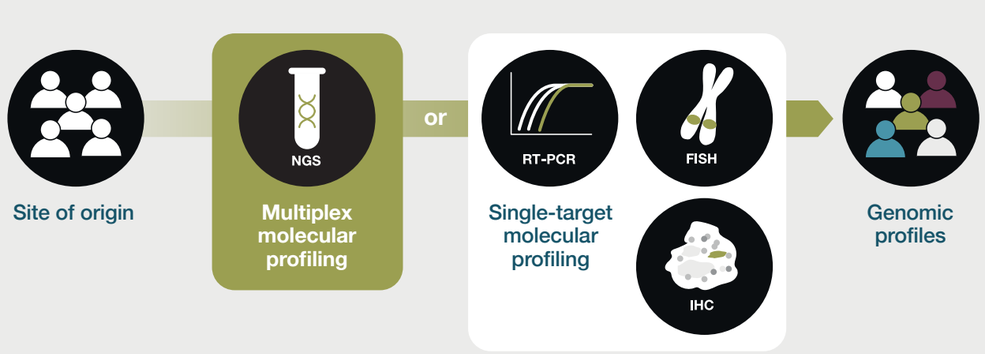
FIGHTING CANCER STARTS BY FINDING ITS FINGERPRINT

Oncology is evolving from thinking about cancer according to site of origin to thinking about cancer according to tumor genomics[1-9]

Tumors can have distinct histologies, sites of origin, and genomic signatures. Over the course of the past decade, the understanding of the centrality of tumor genomics has been increasingly driving oncology, including disease classification, patient selection, and clinical trial design.[7] As the proportion of new FDA-approved treatments classified as personalized medicines increased by 5X between 2005 and 2019, tumor genomics has become ever more critical in treatment selection.[7,8]

Personalized cancer therapy

The prevention, diagnosis, and management of cancer is being revolutionized by precision oncology, which defines cancer by underlying genomic alterations.[1-10]



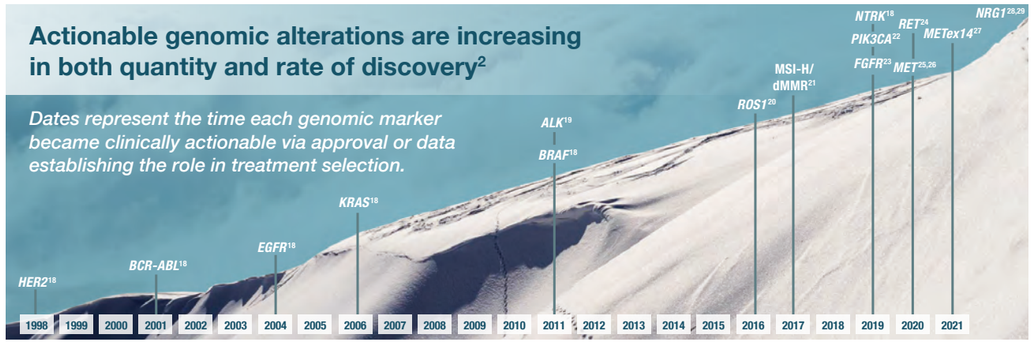
The goal of precision oncology is to optimize and tailor each patient's treatment approach based on the genomic profile of the patient's cancer.[6]

FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; RT-PCR, reverse transcription-polymerase chain reaction.

HOW ARE GENOMICS CHANGING THE FUTURE OF ONCOLOGY?

Understanding the genomic and oncogenic drivers of a patient's cancer can help clinicians develop a more tailored approach to care[5-7,10]

As the understanding of cancer biology has advanced, both the quantity and rate of discovery of genomic alterations have accelerated.[2] In response, investigators are meeting the demand for ways to target them.[2-8,11] More recent studies have estimated higher percentages of actionable alterations, which are only expected to increase as new molecular entities are developed.[8,12-17]



MSI-H/dMMR, microsatellite instability-high/mismatch repair deficiency.

Individual genomic alterations may be rare, but alterations in totality are found in a significant percentage of patients with cancer[12-17]

While treatments are still being developed, it is estimated that >50% of patients may have an actionable genomic alteration.[12-17]

Large retrospective series have documented that up to 90% of patients tested will have potentially actionable alterations. A genomic alteration is typically defined as actionable when there is a potential therapeutic target that can mitigate the oncogenic consequences of the disrupted pathway; although across clinical studies, the definition of actionable can vary substantially.[12,17]

Point mutations and pathogenic gene fusions are among the most common genomic alterations driving cancer[30]

Point mutations (eg, *KRAS*, *BRAF*, *EGFR*) are changes in DNA base pairs.[2,31]

Pathogenic gene fusions (eg, *ALK*, *NTRK*, *ROS1*, *MET*, *RET*, *NRG1*) typically occur when 2 different genes join to form an abnormal hybrid gene.[2,32,33] Genes involved in fusions are not located next to one another but are from separate chromosomal loci.[34] Gene fusions can be comprised of multiple fusion partners.[35]

PRECISION ONCOLOGY PROVIDES A UNIQUE OPPORTUNITY TO IMPROVE CLINICAL OUTCOMES[1-12,17,33,36-40]

Precision oncology benefits

Precision oncology benefits have been reported to potentially include significant improvements in objective response rate (ORR), overall survival (OS), progression-free survival (PFS), and quality of life (QOL) for certain well-characterized molecular alterations with approved targeted therapies compared with conventional chemotherapies approved for the same or overlapping indication and line of therapy.[1-5,7-10,12] With improved outcomes, patients may potentially be able to avoid cycles of trial and error, as well as adverse physical and financial impacts from the cumulative effects of multiple rounds of conventional therapies.[8]

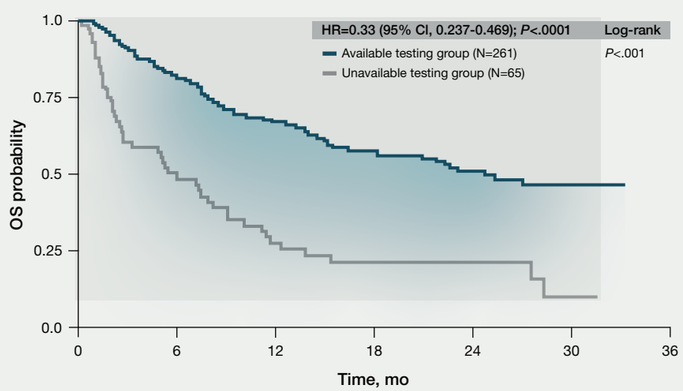
From 2006 to 2018, there was a 7x increase in the number of patients estimated to benefit from genome-based therapy.[38]

In both of the following studies on NSCLC and pancreatic cancer, OS was improved in patients who received therapies directed toward their specific alterations.[39,41]

Overall survival in mNSq NSCLC

A real-world, retrospective, comparative cohort study was conducted using electronic health records of treatment-naive patients newly diagnosed with mNSq NSCLC who had >2 office visits at the University of Pennsylvania's academic practice or 2 affiliated community practices between January 1, 2019, and December 31, 2020. Patients were categorized into the available testing group, which included patients with molecular genotyping results available before the initiation of first-line therapy, or the unavailable testing group, which included patients without molecular genotyping results available before the initiation of first-line therapy. Tissue genotyping was performed using an in-house DNA-based NGS panel along with a targeted RNA sequencing fusion panel. Of 326 patients, 80% (261/326) were in the available testing group and 20% (65/326) were in the unavailable testing group. Of the clinically actionable mutations detected, approximately 70% (103/148) were considered targetable. In the unadjusted Cox proportional-hazards model, with a 14.2-month median follow up, patients in the available testing group had significantly longer OS compared with the unavailable testing group (HR=0.33; 95% CI, 0.237-0.469; *P*<.0001).[41]

OS based on availability of molecular test results before 1L therapy (mNSq NSCLC)[41,a]



1L, first-line; HR, hazard ratio; mNSq, metastatic non-squamous; NSCLC, non–small cell lung cancer.
[a]Retrospective data collected between January 1, 2019, and December 31, 2020.

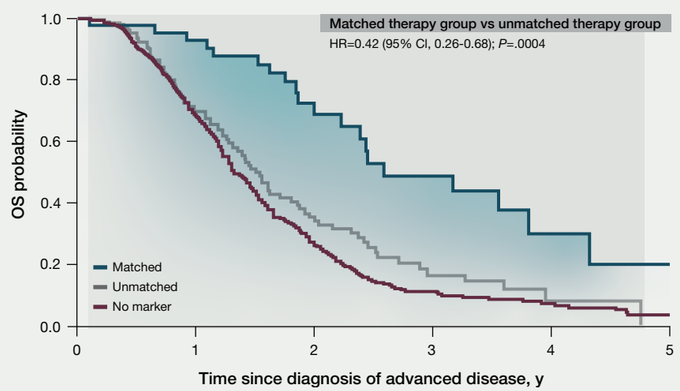
Overall survival in pancreatic cancer

Of 1856 patients with pancreatic cancer who were referred to the Know Your Tumor (KYT) program between June 16, 2014, and March 31, 2019, 1082 (58%) received personalized reports based on their molecular testing results.[39]

With a median follow-up of 383 days (IQR, 214-588), patients with actionable molecular alterations who received a matched therapy (n=46) had significantly longer median OS than patients who only received unmatched therapies (n=143; 2.58 years [95% CI, 2.39 to not reached] vs 1.51 years [95% CI, 1.33-1.87], respectively; HR=0.42 [95% CI, 0.26-0.68]; *P*=.0004).[39]

The 46 patients who received a matched therapy also had longer OS than the 488 patients who did not have an actionable molecular alteration (2.58 years [95% CI, 2.39 to not reached] vs 1.32 years [95% CI, 1.25-1.47], respectively; HR=0.34 [95% CI, 0.22-0.53]; *P*<.0001). Median OS did not differ between patients who received unmatched therapy and those without an actionable molecular alteration (HR=0.82 [95% CI, 0.64-1.04]; *P*=.10).[39]

OS in pancreatic cancer with matched vs unmatched therapy[39]



IQR, interquartile range.

THE CLINICAL CONSEQUENCES OF PATHOGENIC GENE FUSIONS

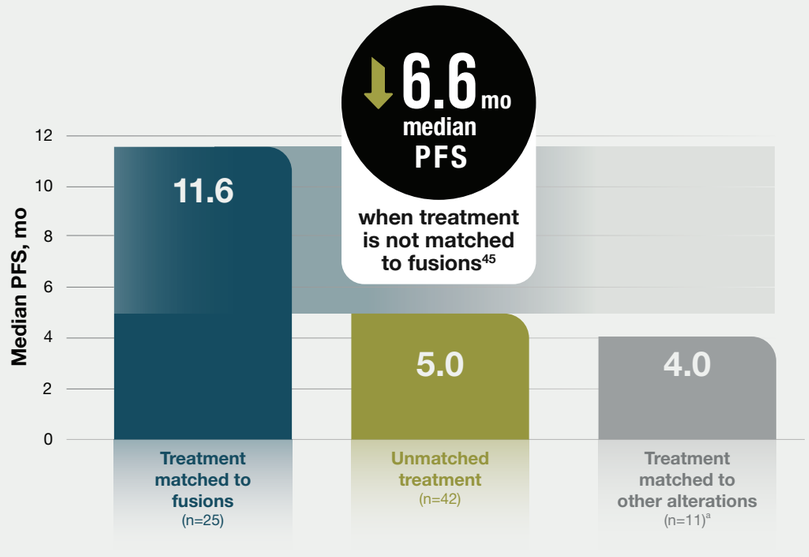
Pathogenic gene fusions are a contributing factor in 1 in 6 cancers[40]

Among 9624 patients who had their tumors genetically tested with RNA-based sequencing, pathogenic gene fusions were found in 16.5% of samples.[40] Fusions can occur across tumor types and account for approximately 20% of cancer morbidity.[33,40,42-45]

Pathogenic gene fusions are an independent poor prognostic factor

A study of 594 patients with fusion-driven lung cancer measured outcomes over time. Patients with a high number of fusions had shorter median overall survival (35.6 months; 95% CI, 27.2-43.9) compared with patients with an intermediate (49.5 months; 95% CI, 23.9-75.1) or low number of fusions (62.3 months; 95% CI, 44.6-80.1; likelihood ratio test, *P*=.008). This relationship persists even when controlled for factors such as age, sex, stage, cancer type, and smoking status.[44]

In an analysis of 79 patients with identified gene fusions, poorer outcomes were observed in patients with pathogenic gene fusions who were not matched to an FDA-approved fusion-targeted therapy.[45]



	Median PFS, mo (95% CI)	PFS ≥6 mo
Matched to fusions (n=25)	11.6 (4.0-35.4)	71.7%
Matched to other alterations (n=11)[a]	4.0 (3.3-NE)	30.3%
Unmatched (n=42)	5.0 (3.3-8.8)	44.6%

[a]Twelve of the 79 patients received treatment matched to other alterations, but 1 patient in the matched group had an unclear match and was excluded from the pairwise comparison analysis.[45]

NRG1: A DANGEROUS PATHOGENIC GENE FUSION

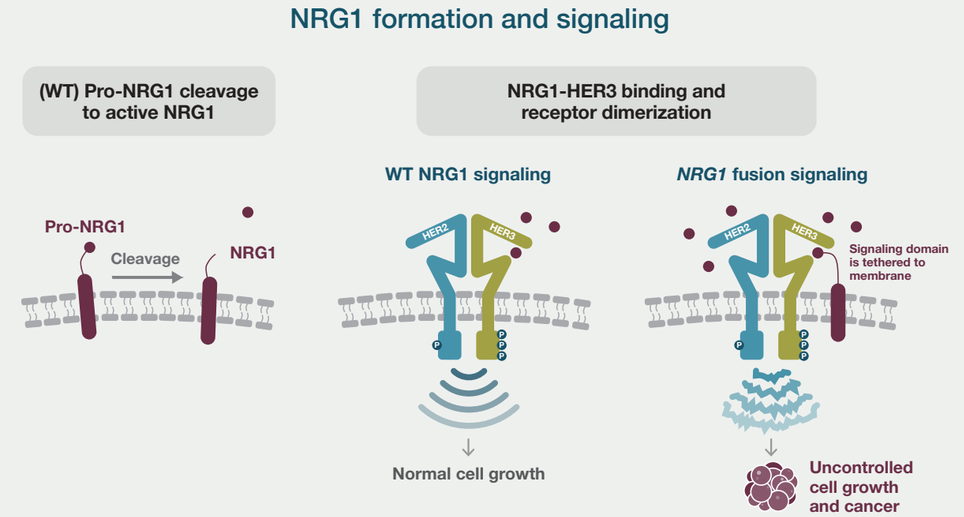
A pathogenic gene fusion receiving increasing attention is *NRG1*, which has been associated with aggressive features and poor outcomes[32,44,46-49]

NRG1 is a key signaling protein involved in proliferation and survival. Normal NRG1 signaling is tightly controlled.[43,46] NRG1 is normally inactive until it is cleaved by proteases at the cell surface. Extracellular binding of NRG1 activates tightly regulated cell growth pathways, including PI3K, AKT, and mTOR. When these pathways are dysregulated, they are capable of becoming oncogenic drivers.[46,47]

Abnormal *NRG1* fusions can lead to uncontrolled growth and cancer.[28,29,43] They can induce the formation of heterodimers, leading to the pathologic activation of signaling pathways and abnormal cell proliferation.[46,49]

NRG1 fusions are heterogenous and can have many different partners and breakpoints.[32,46,47] *NRG1*+ tumors possess histologic features associated with growth, recurrence, invasiveness, metastasis, resistance to therapy, and worse prognosis.[9,32,44,46-48] They respond poorly to available therapies and are associated with lower OS, DFS, and PFS.[9,32,33,36-40,42-49]

Pathogenic *NRG1* fusions are capable of driving cancer growth[43,44,46,47]



Pro-NRG1 is cleaved to active *NRG1*, allowing it to bind to and promote receptor dimerization and signaling.[46]

NRG1 fusions remain anchored in the cell membrane where they bind to and activate HER3, leading to dimerization with HER2 and downstream oncogenic signaling.[50]

DFS, disease-free survival; HER2, human epidermal growth factor receptor 2; HER3, human epidermal growth factor receptor 3; NE, not estimable; *NRG1*+, neuregulin 1 fusion positive; WT, wild type.

NRG1 ACROSS TUMOR TYPES

NRG1 gene fusions have been identified across many tumor types and generally occur in the absence of other driver mutations[32,43,46]

NRG1 fusion frequency estimates

Overall (0.3%-1.7%)[51,52]

Enrichment

Invasive mucinous lung adenocarcinoma (27%-31%)[43,46]

Overall (0.5%-1.8%)[43,53]

Enrichment

KRAS wild-type pancreatic cancer (up to 6%)[48]

Overall (<1%)[43]

Enrichment

Breast, cholangiocarcinoma, colorectal, gallbladder, sarcoma, ovarian cancers, renal cell carcinoma, etc[43,47]

NRG1 fusions are associated with poor outcomes and resistance to standard therapies[32]

In a retrospective global registry study, *NRG1*+ NSCLC was associated with limited response to available therapies. Of 110 patients with *NRG1*+ lung cancer included in the eNRGy1 global multicenter registry, 103 had adenocarcinoma, of which 59 (57%) were IMA, 29 (28%) were nonmucinous, and 15 (15%) were "other" or "unspecified."[32]

Activity of systemic therapy in *NRG1*+ NSCLC[32,a]	ORR, %	Median PFS, mo (95% CI)
Platinum-doublet chemotherapy (n=15)	**13**	**5.8** (2.2-9.8)
Taxane-based chemotherapy (n=7)	**14**	**4.0** (0.8-5.3)
Combination chemotherapy and immunotherapy (n=9)	**0**	**3.3** (1.4-6.3)
Single-agent immunotherapy (n=5)	**20**	**3.6** (0.9-undefined)
Targeted therapy with kinase inhibitor (n=20)	**25**	**2.8** (1.9-4.3)

[a]Patients either diagnosed with or who developed metastatic disease during the course of their disease.[32]

AGGRESSIVE HISTOLOGICAL FEATURES

NRG1 fusions have aggressive histological features

Chang et al conducted a molecular and clinicopathologic analysis of 200 cases of pulmonary IMA diagnosed between 2009 and 2019. Genomic analysis was conducted using hotspot mutation testing, targeted DNA sequencing, and targeted RNA sequencing. The investigators found that 92% of the IMA tumors that were *NRG1*+ possessed aggressive histological features associated with poor outcomes compared with 54% of *KRAS*+ tumors and 61% of tumors with other driver alterations.[54,b] Findings were consistent with other studies suggesting that *NRG1*+ lung and gastric tumors are associated with increased infiltrative tumor growth, as well as lymphovascular, neural, and desmoplasmic stromal invasion, which are associated with poor outcomes.[47]

Growth

In the same study, Chang et al also measured primary tumor size pathologically in resected tumors and radiologically in unresected tumors. Among all tumors tested, gene fusions were identified in a total of 24 IMAs, including 12 (50%) with *NRG1*, 6 (25%) with *ALK*, 2 (8%) with *ROS1*, and 1 each with *ERBB2*, *NTRK1*, *FGFR2*, and *FGFR3*. The investigators found dramatically increased primary tumor size at diagnosis for *NRG1*+ vs *KRAS*+ and other IMA tumors (7.7 cm vs 3.9 cm vs 5.5 cm, respectively; *P*=.0004).[b] This study documented more aggressive histological and clinical characteristics of IMAs with *NRG1* fusions. The presence of these characteristics has been found to correlate with worse prognosis for patients with IMA.[54]

Migration

Shin et al studied a cohort of 59 patients with IMA who underwent curative surgical resection, 16 of whom had *NRG1* fusions. The majority of cases with *NRG1*+ samples had pathological stage I disease. Investigators found that an *SLC3A2-NRG1* fusion promoted increased tumor volume, as well as cancer cell proliferation and migration, using a shedding and juxtacrine method through *ERBB2-ERBB3* heterocomplexes. This association strengthened with increased *NRG1* fusion protein expression.[c] Cancer cell migration induced by the *SLC3A2-NRG1* fusion protein was due to an increase in pFAK and pSrc by the *SLC3A2-NRG1* fusion protein—this was not induced by *SLC3A2-NRG1*Δ EGF. Results indicated that the EGF domain in the *NRG1* part of the *SLC3A2-NRG1* fusion augmented cell proliferation and migration.[9]

EGF, epidermal growth factor; IMA, invasive mucinous adenocarcinoma.

[b]In a study by Chang et al (2021), samples from 200 IMA cases were reviewed by 2 thoracic pathologists. Primary tumor size was measured pathologically in resected tumors and radiologically in unresected tumors. Presence of tumor necrosis and stromal invasion, defined by stromal desmoplasia surrounding invasive glands or nests of tumor cells, were recorded.[54]

[c]Shin et al (2016) tested 59 IMA samples obtained from patients who underwent curative surgical resection, identifying 13 *SLC3A2-NRG1* fusions (27% frequency). Tumor xenografts in nude mice were generated for measuring tumor volume and tumor weight. Tumor proliferation, volume, and weight were analyzed in cancer cells ectopically expressing *SLC3A2*, *NRG1*, and *SLC3A2-NRG1*.[9]

UNIQUE PATTERNS OF METASTASIS

Metastatic potential

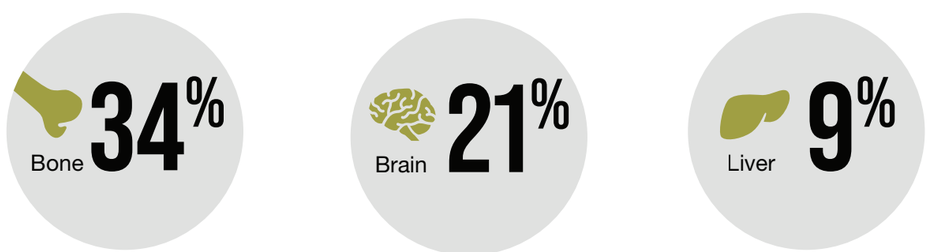
IMA has been reported to represent 3% to 5% of adenocarcinomas overall. A recent study evaluated the histology and genomic profiles of tissue samples from 200 cases of IMA. *KRAS* alterations were identified in 151 of the IMA samples, and gene fusions were identified in 24 samples. Half of the fusions (12) were *NRG1+*. *NRG1* fusions were associated with significantly lower cigarette exposure compared with *KRAS* fusions (5.9 vs 20 pack-years, respectively). Presence of metastasis at diagnosis, as well as the frequency of extrathoracic metastases, were higher for *NRG1* vs *KRAS*.[54]

NRG1+ tumors have a higher rate of metastasis at diagnosis compared with *KRAS+* tumors (67% vs 32%, respectively)[54]

NRG1+ tumors are 10x more likely to have concurrent intra- and extrathoracic metastases than *KRAS+* tumors (50% vs 5%, respectively)[54]

Similar results were observed in a study by Drilon et al about the clinicopathologic features of *NRG1* fusion–driven lung cancers, in which data were collected from a consortium of 22 centers from 9 countries. At the time of diagnosis, most (71%, n=58/82) patients had nonmetastatic (stages I-III) disease. In patients with metastatic *NRG1*-driven disease diagnosed at any time during their disease course (n=44), extrathoracic metastases were found in 43% (n=19/44) of patients.[32]

Most common sites of *NRG1+* extrathoracic metastases in IMA[32]



THE EVOLUTION OF GENOMIC TESTING

Conventional testing methods

RT-PCR, FISH, and IHC are biomarker screening methods that were developed to detect single molecular targets and may fall short of detecting pathogenic gene fusions.[42,55,56]

Specifically, limitations include:

- Inability to identify the full breadth of genomic alterations[42,57]
- Limited ability to identify the full breadth of fusion partners and breakpoints[42,56]
- May require a significant amount of tissue and can exhaust tissue samples[58]

The advent of next-generation sequencing

Since the completion of the National Human Genome Project was announced in 2003, genome sequencing technology has improved dramatically. In particular, the decade that followed saw revolutionary advances in sequencing technologies that fundamentally changed the nature of genomics. The advent of “next-generation” sequencing in 2008 welcomed significant improvements in both accuracy and efficiency, bringing with it a rapid reduction in costs and turnaround time.[59]

NEXT-GENERATION SEQUENCING CAN DETECT A BROAD RANGE OF GENOMIC ALTERATIONS[2,5,55,60]

NGS has emerged as a key tool in profiling many solid tumors[60]

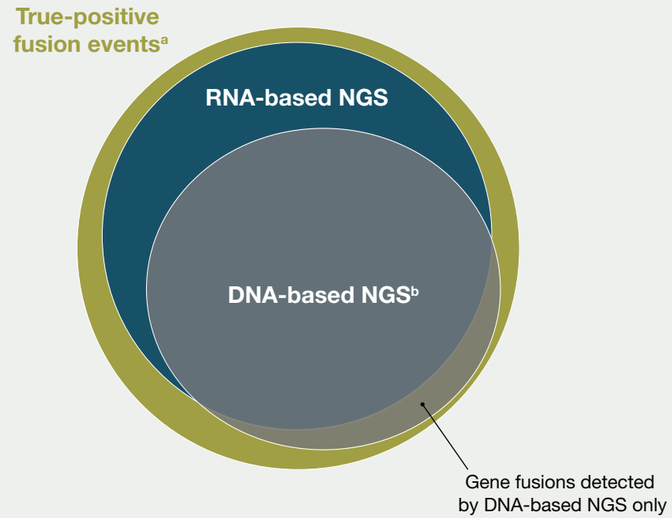
NGS is a high-throughput genomic sequencing technology that allows for the simultaneous analysis of numerous alterations; NGS can be DNA-based, RNA-based, or both[55,61]

NGS is a young field, with the first machines entering the market less than 2 decades ago. In less than a decade, NGS became a cornerstone of molecular biology and genetics. More recently, NGS systems have been introduced that allow for massively parallel sequencing reactions. These systems are capable of analyzing millions, or even billions, of sequencing reactions at the same time, dramatically increasing the efficiency of sequencing genomes. Unlike some tools, NGS is flexible and can be applied in different situations, ranging from exome to small RNAs.[6,62,63]

The NGS process[62]

DNA-BASED NGS ALONE CAN MISS PATHOGENIC GENE FUSIONS[64,65]

Comprehensive testing with RNA-based NGS, including DNA and RNA sequencing, is recommended to capture what DNA-based NGS alone can miss[55,64]



DNA-based sequencing can lead to false-negative and false-positive results in a variety of cases, particularly in the detection of gene fusions.[42,55,64-66]

Comprehensive genomic sequencing—a more efficient option that sequences both RNA and DNA simultaneously—should take place at diagnosis, or as early as possible in the course of disease, to maximize the range of treatment options available to patients.[36,66]

[a]Graphic for illustrative purposes only. Not drawn to scale or reflective of actual results captured by different methodologies.
[b]DNA-based NGS can detect some fusions not found by certain RNA-based NGS.[65]

WHY IS RNA-BASED NGS MORE COMPREHENSIVE FOR DETECTING PATHOGENIC GENE FUSIONS?[64-69]

Advantages of RNA-based NGS

- Detects gene expression and many structural variants[2,42,64,67]
- Reduces many of the technical challenges involved in sequencing long introns[55,64-67,69]
- Can improve the detection rate of DNA-based NGS alone and provide more comprehensive detection results[16,64-69]
- May enable oncologists to match therapy to the driving fusion, which wouldn't have otherwise been identified, potentially leading to improved clinical responses[16]

RNA-based NGS for detecting pathogenic gene fusions	RNA	DNA
Overcomes difficulties caused by large introns[54,63-66,68]	✓	✗
Facilitates realignments in intron repeats[65,68]	✓	✗
Assay sensitivity is retained with low tumor sample if highly expressed[42,63,68]	✓	✗
Captures a broad range of complex genomic events[2,42,68]	✓	✗

RNA-BASED NGS IS ESSENTIAL FOR OPTIMIZING DETECTION OF MANY *NRG1* FUSIONS[64-69]

Detecting *NRG1* pathogenic gene fusions

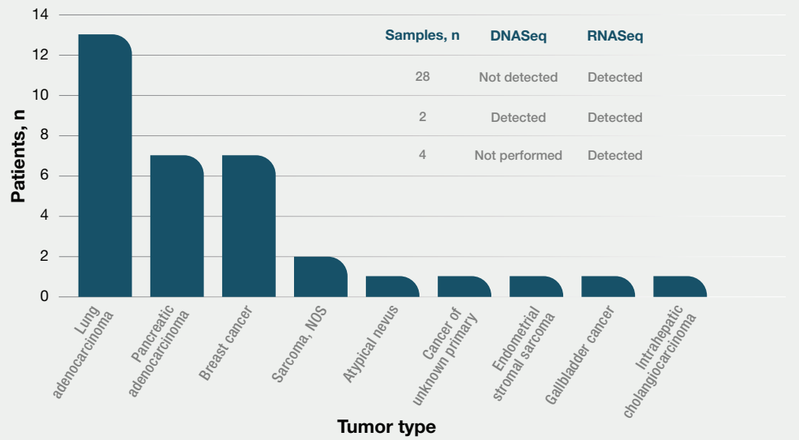
- *NRG1* fusions are more likely to be missed without the use of RNA-based NGS[64-69]
- The diversity of *NRG1* fusion partners and breakpoints and the large intronic regions of the *NRG1* gene can make detection more challenging[55,64-67,69]

In a retrospective study by the Memorial Sloan Kettering Cancer Center,

RNA-based NGS detected more *NRG1* fusions than DNA-based NGS[66]

Both DNA-based and RNA-based NGS were performed on 30 *NRG1*+ IMA samples. Of these, 28 were detected by RNA-based NGS but not DNA-based NGS. The remaining 2 were detected by both. Four additional samples that did not undergo DNA-based NGS were detected by RNA-based NGS.[66]

Detection of *NRG1* fusions across tumor types (MSKCC experience)[66]



Of the 60,000 tumor specimens that have undergone molecular profiling by DNA-based NGS at MSKCC, *NRG1* fusions were detected at a rate of just 20% of the estimated prevalence in the population. This further indicates that DNA-based NGS by itself is not the optimal approach for the comprehensive detection of *NRG1* fusions. It can be a challenge to detect *NRG1* fusions with standard assays, and the tests that can detect them are not always performed.[66]

MSKCC, Memorial Sloan Kettering Cancer Center; NOS, not otherwise specified.

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INCOMPLETE KNOWLEDGE HAS CONSEQUENCES

Detecting pathogenic gene fusions is critical[1,32,33,40,42,45,55,64]

Oncology is evolving from thinking about cancer according to site of origin to thinking about cancer according to tumor genomics.[1-9]

- Pathogenic gene fusions are becoming increasingly actionable[45,46]
- Targeting these genomic alterations may potentially lead to improved outcomes[1,40,42,45,55,64,65]

***NRG1* is an important pathogenic gene fusion** that can occur across tumor types and is associated with poor outcomes and resistance to standard therapies.[9,32,46-49,51,54,66]

RNA-based NGS is capable of supporting broader identification of genomic alterations, including pathogenic gene fusions such as *NRG1*, when compared with DNA-based methods.[32,33,42,43,55,64-69]

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