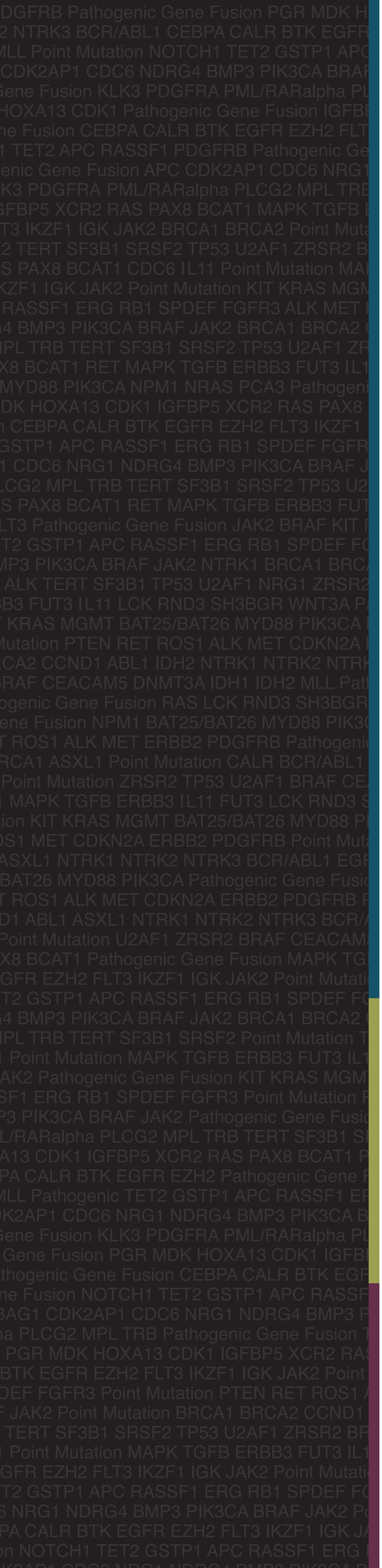

SIMPLIFYING YOUR APPROACH TO PRECISION ONCOLOGY

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

PTx® PARTNER THERAPEUTICS®

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The Promise of Precision Oncology

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

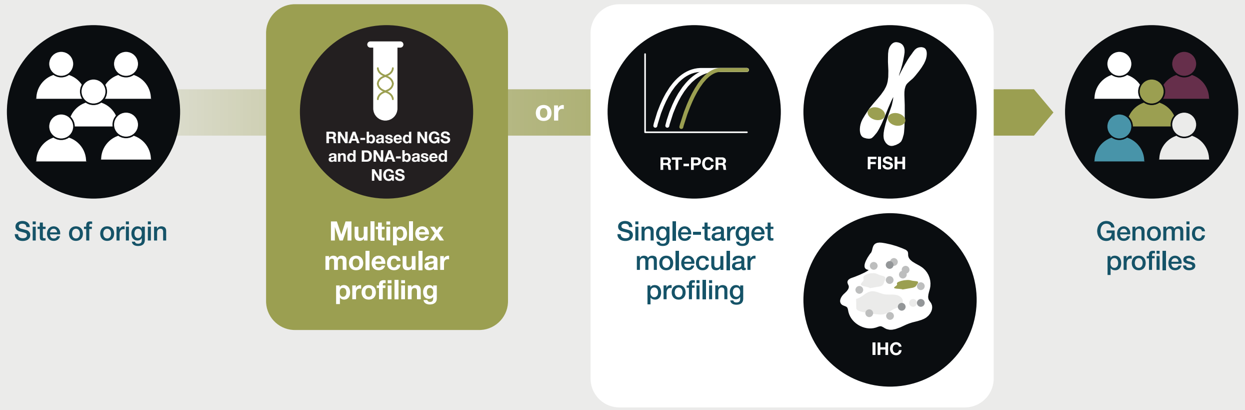
FIGHTING CANCER STARTS BY FINDING ITS FINGERPRINT

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] With 18 new FDA-approved personalized treatments in 2024, these therapies comprise at least a quarter of all new drug approvals annually for the past decade. Just over 10 years ago, they represented less than 10% of approvals. This rapid growth underscores the increasingly central role of tumor genomics in guiding treatment decisions.[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]



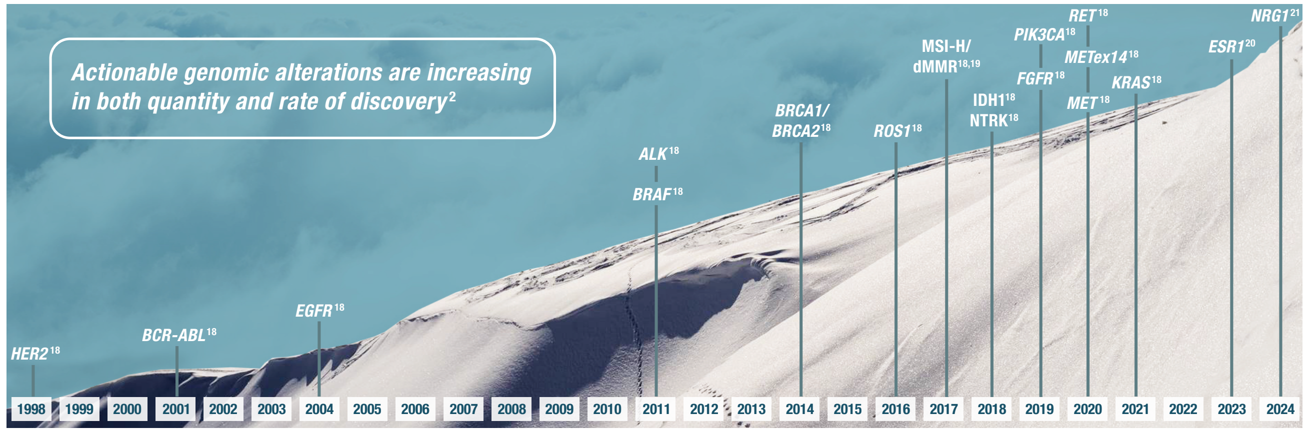
The goal of precision oncology is to optimize and tailor each patient's treatment approach based on the genomic profile of the patient's cancer.[6]

FDA, Food and Drug Administration; 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 ~38% of patients have an actionable genomic alteration.[22]

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[23]

Point mutations (eg, *KRAS*, *BRAF*, *EGFR*) are changes in DNA base pairs.[2,24]

Pathogenic gene fusions (eg, *ALK*, *NTRK*, *ROS1*, *RET*, *NRG1*) typically occur when 2 different genes join to form an abnormal hybrid gene.[2,25,26] Genes involved in fusions are not located next to one another but are from separate chromosomal loci.[27] Gene fusions can be comprised of multiple fusion partners.[28]

PRECISION ONCOLOGY PROVIDES A UNIQUE OPPORTUNITY TO IMPROVE CLINICAL OUTCOMES[1-12,17,26,29-33]

Targeting genomic alterations can lead to better outcomes for patients

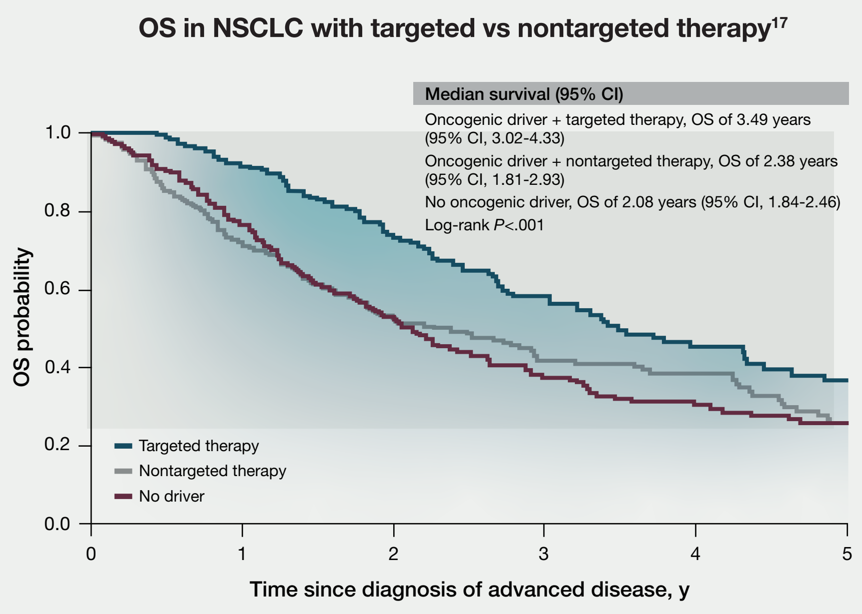
Precision oncology benefits have been reported to potentially include significant improvements in objective response rate (ORR), overall survival (OS), progression-free survival (PFS), and quality of life (QOL) for certain well-characterized molecular alterations with approved targeted therapies compared 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.[31]

In both of the following studies on NSCLC and pancreatic cancer, patients who received therapies directed towards their specific alteration lived longer.[17,32]

Overall survival in NSCLC

In a study of 938 patients with metastatic lung adenocarcinomas and a Southwest Oncology Group (SWOG) performance status of 0 through 2, tumors were tested for 10 oncogenic drivers. The study collected information from patients, therapies, and OS. Three-hundred sixty (38%) patients had no identified oncogenic driver, while 578 (62%) had actionable oncogenic drivers. Of those with actionable oncogenic drivers, decision-making in cancer care for 318 patients (55%) was not impacted, while 260 patients (45%) had identified drivers that impacted cancer management. Patients who received a matched therapy for their actionable molecular alterations had longer OS of 3.49 years (95% CI, 3.02-4.33) vs 2.08 years (95% CI, 1.84-2.46) for those with no known oncogenic drivers (log-rank *P*<.001).[17]



OS in NSCLC with targeted vs nontargeted therapy[17]

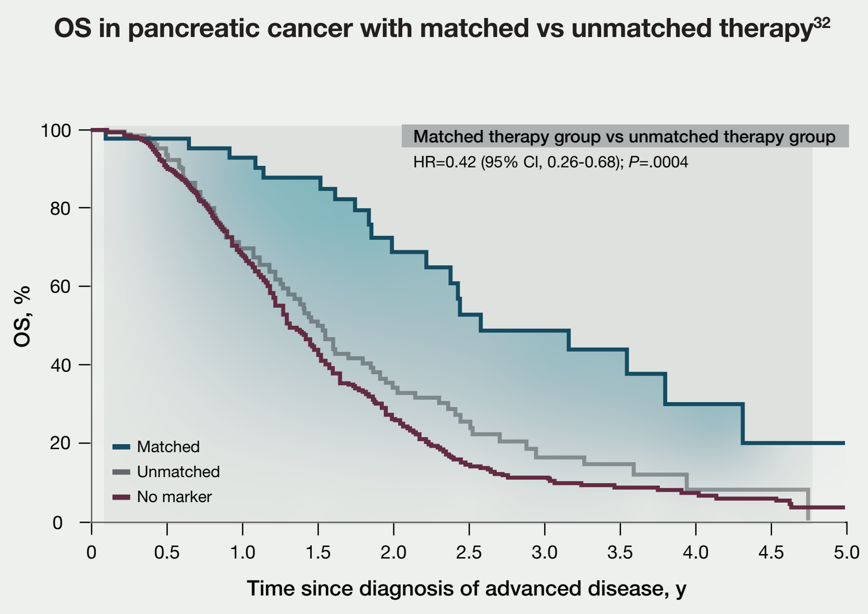
NSCLC, non–small cell lung cancer.

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.[32]

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).[32]

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).[32]



OS in pancreatic cancer with matched vs unmatched therapy[32]

IQR, interquartile range.

THE CLINICAL CONSEQUENCES OF PATHOGENIC GENE FUSIONS

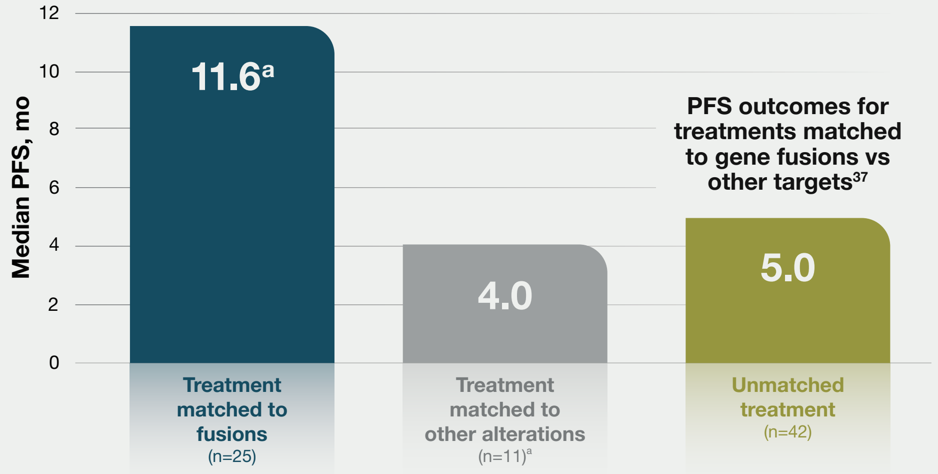
Pathogenic gene fusions are a contributing factor in 1 in 6 cancers[33]

Among 9624 patients who had their tumors genetically tested with RNA-based sequencing, pathogenic gene fusions were found in 16.5% of samples.[33] Fusions can occur across tumor types and account for approximately 20% of cancer morbidity.[26,33-37]

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 OS (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.[36]

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.[37]



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[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.[37]

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[25,36,38-41]

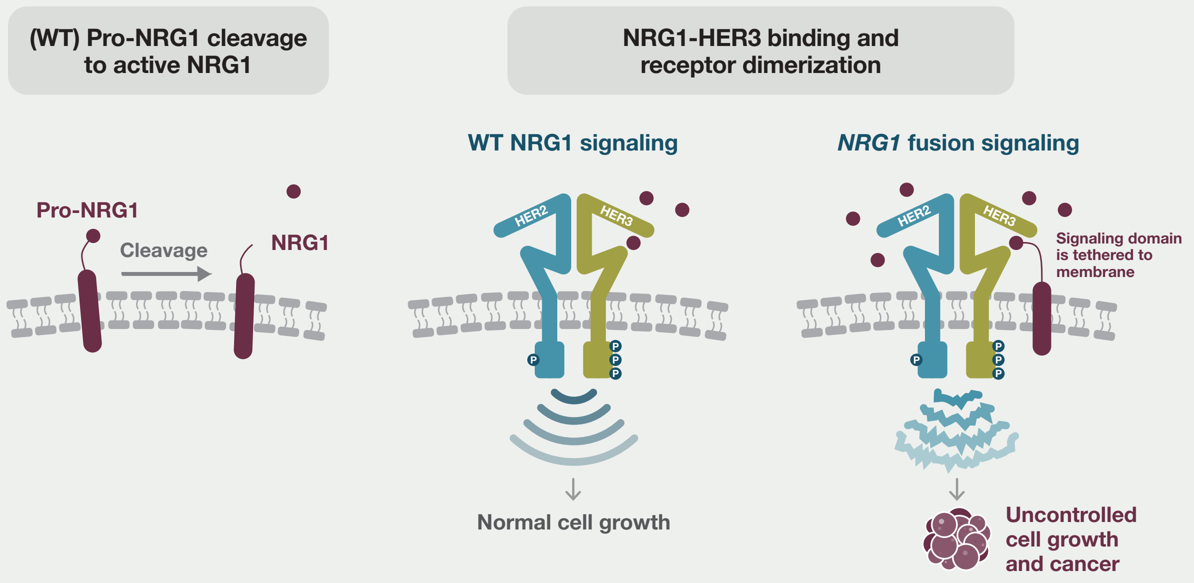
NRG1 is a key signaling protein involved in proliferation and survival. Normal NRG1 signaling is tightly controlled.[35,38] NRG1 is normally inactive until it is cleaved by proteases at the cell surface. Extracellular binding of NRG1 activates tightly regulated cell growth pathways, including PI3K, AKT, and mTOR. When these pathways are dysregulated, they are capable of becoming oncogenic drivers.[38,39]

Abnormal *NRG1* fusions can lead to uncontrolled growth and cancer.[35] They can induce the formation of heterodimers, leading to the pathologic activation of signaling pathways and abnormal cell proliferation.[38,41]

NRG1 fusions are heterogenous and can have many different partners and breakpoints.[25,38,39] *NRG1*+ NSCLC possesses histologic features associated with growth, recurrence, invasiveness, metastasis, resistance to therapy, and worse prognosis.[9,25,36,38-40] *NRG1*+ NSCLC responds poorly to available therapies and is associated with lower OS, DFS, and PFS.[9,25,26,29-41]

Pathogenic *NRG1* fusions are capable of driving cancer growth[35-38]

NRG1 formation and signaling



Pro-NRG1 is cleaved to active *NRG1*, allowing it to bind to and promote receptor dimerization and signaling.[38]

NRG1 fusions remain anchored in the cell membrane where they bind to and activate HER3, leading to dimerization with HER2 and downstream oncogenic signaling.[42]

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 solid tumors and generally occur in the absence of other driver mutations[25,35,38]

NRG1 fusion frequency estimates

Lung cancer

Overall (<1%)[42,43]

Enrichment

Invasive mucinous lung adenocarcinoma (up to 31%)[9,44,45]

Pancreatic cancer

Overall (<2%)[43,46]

Enrichment

KRAS wild-type pancreatic cancer (up to 3%)[46,47]

Other

Overall (<1%)[43]

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

NRG1 fusions are associated with poor outcomes and resistance to therapies[25]

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."[25]

Activity of systemic therapy in *NRG1*+ NSCLC[25,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.[25]

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.[44,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.[39]

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.[44]

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.[44]

[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 in *NRG1*+ tumors

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*.[44]

Higher rate of metastasis

NRG1+ tumors have a higher rate of metastasis at diagnosis compared with *KRAS*+ tumors (67% vs 32%, respectively)[44]

10x

NRG1+ tumors are 10x more likely to have concurrent intra- and extrathoracic metastases than *KRAS*+ tumors (50% vs 5%, respectively)[44]

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.[25]

Most common sites of *NRG1*+ extrathoracic metastases in IMA[25]

Bone **34%**

Brain **21%**

Liver **9%**

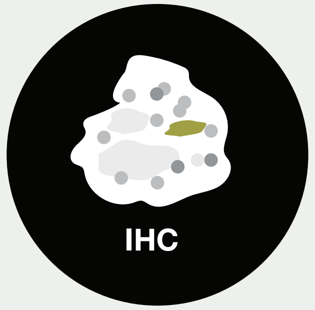
THE EVOLUTION OF GENOMIC TESTING

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.[34,48,49]

Specifically, limitations include:

- Inability to identify the full breadth of genomic alterations[34,50]
- Limited ability to identify the full breadth of fusion partners and breakpoints[34,49]
- May require a significant amount of tissue and can exhaust tissue samples[51]



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.[52]

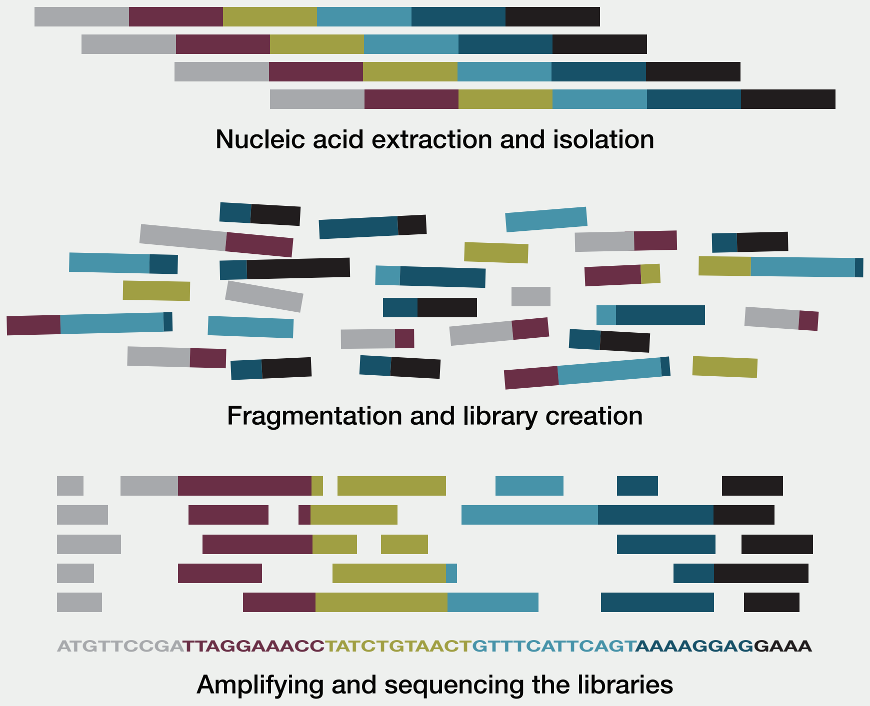
NEXT-GENERATION SEQUENCING CAN DETECT A BROAD RANGE OF GENOMIC ALTERATIONS[2,5,48,53]

NGS has emerged as a key tool in profiling many solid tumors[53]

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[48,54]

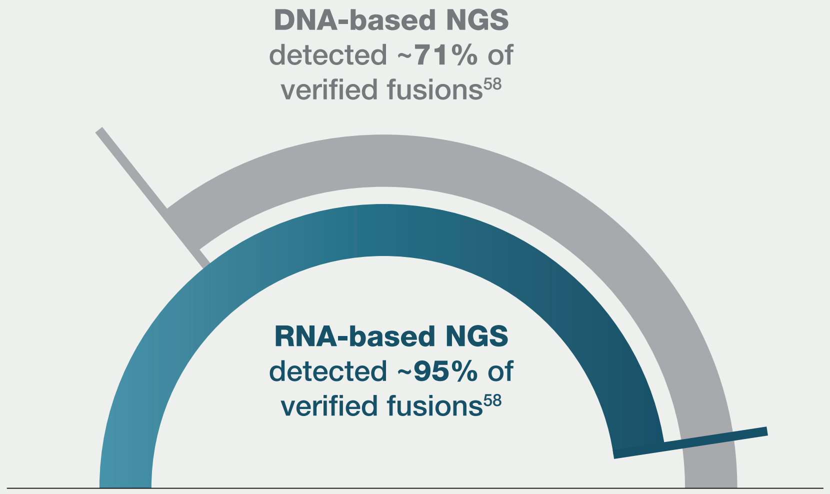
NGS is a young field, with the first machines entering the market less than 2 decades ago. In less than a decade, NGS became a cornerstone of molecular biology and genetics. More recently, NGS systems have been introduced that allow for massively parallel sequencing reactions. These systems are capable 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.[6,55,56]

The NGS process[55]



DNA-BASED NGS ALONE CAN MISS PATHOGENIC GENE FUSIONS[57,58]

Comprehensive testing with RNA-based NGS, including DNA and RNA sequencing, is recommended to capture what DNA-based NGS alone can miss[25,57]



DNA-based NGS detected ~**71%** of verified fusions[58]

RNA-based NGS detected ~**95%** of verified fusions[58]

Together **NGS using both RNA + DNA** detected **all** verified fusions in the study (N=2118 fusion events)[58,a]

DNA-based sequencing can lead to false-negative and false-positive results in a variety of cases, particularly in the detection of gene fusions.[34,48,57-60]

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.[29,60]

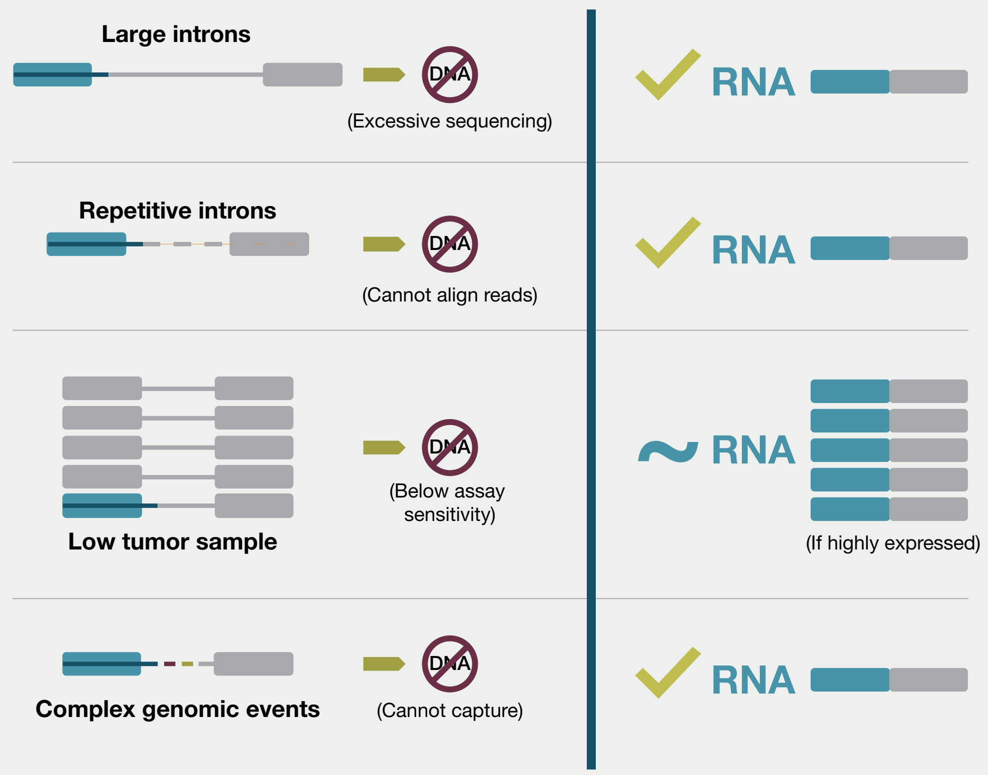
[a] Based on NGS testing of tissue samples.[58]

WHY IS RNA-BASED NGS MORE COMPREHENSIVE FOR DETECTING PATHOGENIC GENE FUSIONS?[57,59-63]

Advantages of RNA-based NGS

- Detects gene expression and many structural variants[1,34,57,61]
- Reduces many of the technical challenges involved in sequencing long introns[48,57,59-63]
- Can improve the detection rate of DNA-based NGS alone and provide more comprehensive detection results[16,57,59-63]
- May enable oncologists to match therapy to the driving fusion, which wouldn't have otherwise been identified, potentially leading to improved clinical responses[16]

DNA-based vs RNA-based NGS for fusions[63]



RNA-BASED NGS IS ESSENTIAL FOR OPTIMIZING DETECTION OF MANY *NRG1* FUSIONS[57,59-63]

Detecting *NRG1* pathogenic gene fusions

- *NRG1* fusions are more likely to be missed without the use of RNA-based NGS[57,59-63]
- The diversity of *NRG1* fusion partners and breakpoints and the large intronic regions of the *NRG1* gene can make detection more challenging[48,57,59-61,63]

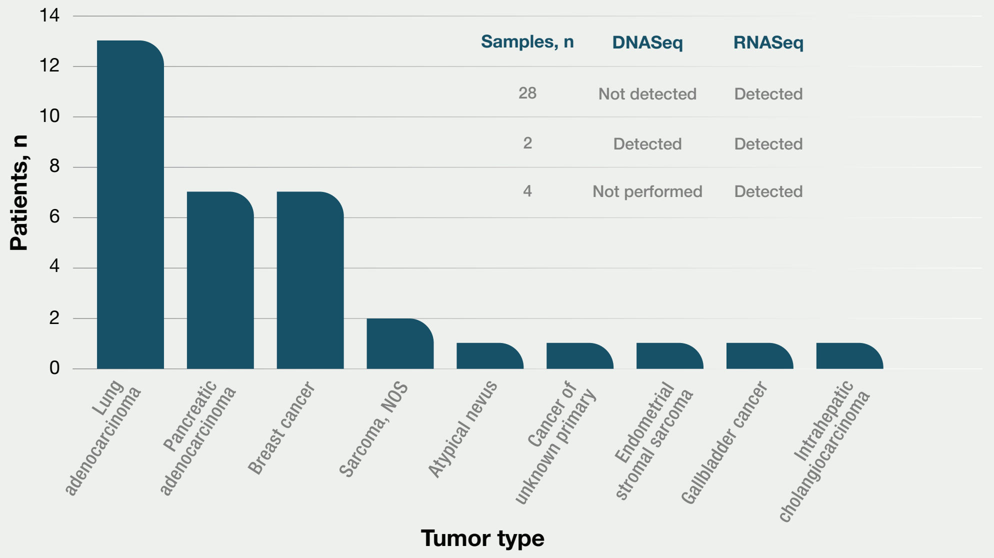
***NRG1* detection**

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

RNA-based NGS detected more *NRG1* fusions than DNA-based NGS[60]

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.[60]

Detection of *NRG1* fusions across tumor types (MSKCC experience)[60]



Samples, n	DNASeq	RNASeq
28	Not detected	Detected
2	Detected	Detected
4	Not performed	Detected

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.[60]

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

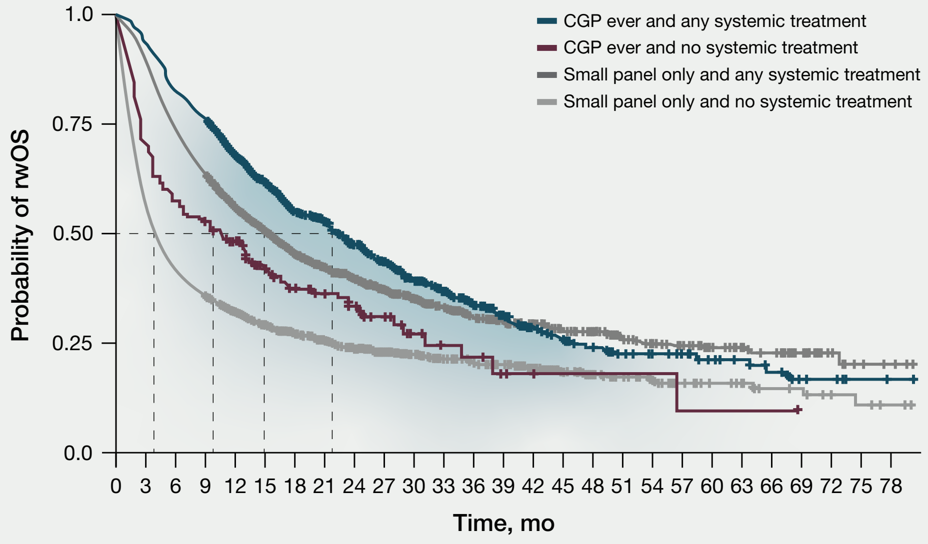
COMPREHENSIVE GENOMIC PROFILING IS ASSOCIATED WITH IMPROVED OUTCOMES[64]

The impact of genomic profiling

A real-world, retrospective study evaluated the clinical impact of CGP vs small panel testing in patients with aNSCLC. Using data from community oncology settings, researchers compared outcomes related to biomarker detection, use of targeted therapies, and rwOS.[64]

Patients tested with CGP were more likely to have actionable biomarkers identified (32% vs 14%; *P*<.001) and to receive matched targeted therapies (39% vs 29%; *P*<.001).[64]

rwOS from aNSCLC diagnosis, by testing type and receipt of systemic therapy[64]



No. at risk at index	Testing/treatment group	Median, mo (95% CI)
1852	Small panel with any systemic therapy	**15** (14-16)
1253	Small panel and no systemic therapy	**4** (4-5)
603	CGP with any systemic therapy	**22** (18-25)
176	CGP and no systemic therapy	**10** (6-15)

Predictors of rwOS by testing type (Cox proportional hazards model)

	Alive (N=1186)	Deceased (N=2698)	HR (95% CI)
CGP testing, No (%)			
No	**906** (76%)	**2199** (82%)	**1.00**
Yes	**280** (24%)	**499** (18%)	**0.80** (0.72-0.89)

Higher median rwOS

Treated patients receiving CGP testing during follow-up had higher median rwOS (22 months vs 15 months)[64]

aNSCLC, advanced non–small cell lung cancer; CGP, comprehensive genomic profiling; HR, hazard ratio; rwOS, real-world overall survival.

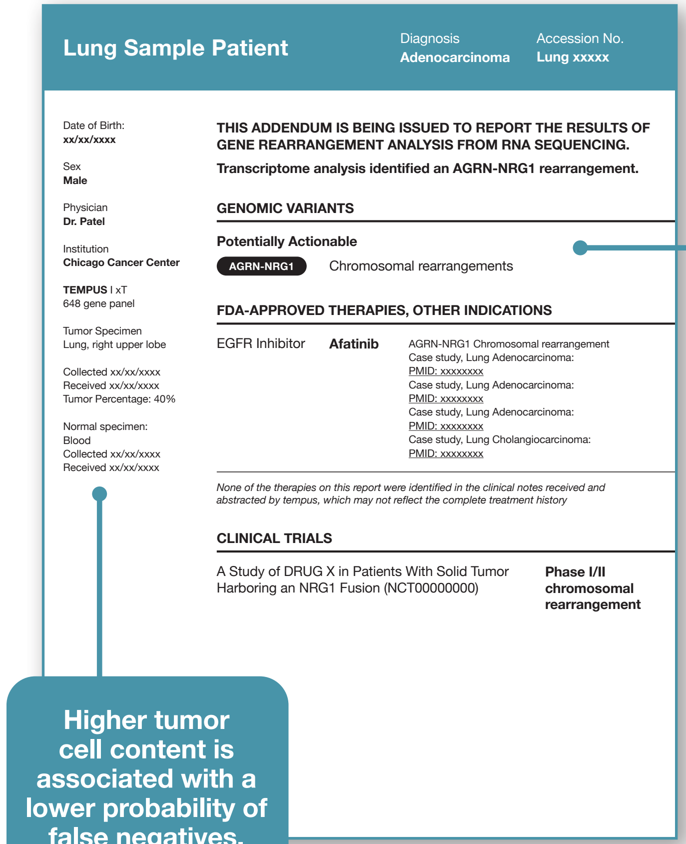
IT IS TIME TO TEST FOR *NRG1* FUSIONS

NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) include RNA-based NGS[65,66]

- **Pancreatic adenocarcinoma:** Tumor/somatic molecular profiling, preferably using NGS assay, is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify clinically actionable and/or emerging alterations. These alterations include, but are not limited to, *NRG1* fusions. RNA sequencing assays are preferred for detecting RNA fusions because gene fusions are better detected by RNA-based NGS[65]
- **NSCLC:** RNA-based NGS may increase novel fusion detection and can be performed concurrently or sequentially with DNA-based NGS. If no identifiable driver oncogenes with DNA-based broad molecular profiling are identified, RNA-based testing is recommended[66]

Detecting *NRG1* fusions through comprehensive testing is a critical step toward optimizing care and potentially improving outcomes[65,66]

Most NGS reports highlight actionable information[67]



Lung Sample Patient

Diagnosis
Adenocarcinoma

Accession No.
Lung xxxxx

Date of Birth:
xx/xx/xxxx

Sex
Male

Physician
Dr. Patel

Institution
Chicago Cancer Center

TEMPUS I xT
648 gene panel

Tumor Specimen
Lung, right upper lobe

Collected xx/xx/xxxx
Received xx/xx/xxxx
Tumor Percentage: 40%

Normal specimen:
Blood
Collected xx/xx/xxxx
Received xx/xx/xxxx

THIS ADDENDUM IS BEING ISSUED TO REPORT THE RESULTS OF GENE REARRANGEMENT ANALYSIS FROM RNA SEQUENCING.

Transcriptome analysis identified an AGRN-NRG1 rearrangement.

GENOMIC VARIANTS

Potentially Actionable

AGRN-NRG1 Chromosomal rearrangements

FDA-APPROVED THERAPIES, OTHER INDICATIONS

EGFR Inhibitor	**Afatinib**	AGRN-NRG1 Chromosomal rearrangement Case study, Lung Adenocarcinoma: PMID: xxxxxxxx Case study, Lung Adenocarcinoma: PMID: xxxxxxxx Case study, Lung Adenocarcinoma: PMID: xxxxxxxx Case study, Lung Cholangiocarcinoma: PMID: xxxxxxxx

None of the therapies on this report were identified in the clinical notes received and abstracted by tempus, which may not reflect the complete treatment history

CLINICAL TRIALS

A Study of DRUG X in Patients With Solid Tumor Harboring an NRG1 Fusion (NCT00000000)	**Phase I/II chromosomal rearrangement**

Most reports will provide variant results listed by a tier of evidence in order of relevance and relevant FDA-approved therapies. Certain reports may identify possible clinical trial options.

Higher tumor cell content is associated with a lower probability of false negatives.

Methodology

Test material: Tumor DNA/RNA[67]

Gene panel: Tempus xT Targeted Panel of 648 genes. Assay v4—a custom oncology testing panel (see detailed list of genes in full report)[68,69]

Instrument: Illumina Novaseq 6000

Reference genome: GRCh37 (hg19)[69]

Methodology identifies material tested (DNA and/or RNA).

Methodology details include test description, sequencing instrument, and reference genome used.

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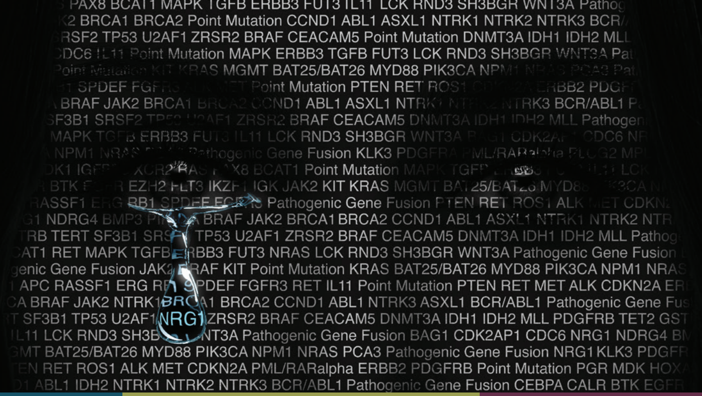
INCOMPLETE KNOWLEDGE HAS CONSEQUENCES



Detecting pathogenic gene fusions in cancer is critical to help improve outcomes for patients[16,33,44]

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[2,18,21]
- Targeting these genomic alterations may potentially lead to improved outcomes[17,32]

***NRG1* is an important pathogenic gene fusion** that can occur across solid tumors and is associated with poor outcomes and resistance to therapies in *NRG1*+ NSCLC.[25,39,43]

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.[48,53,54,57]

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