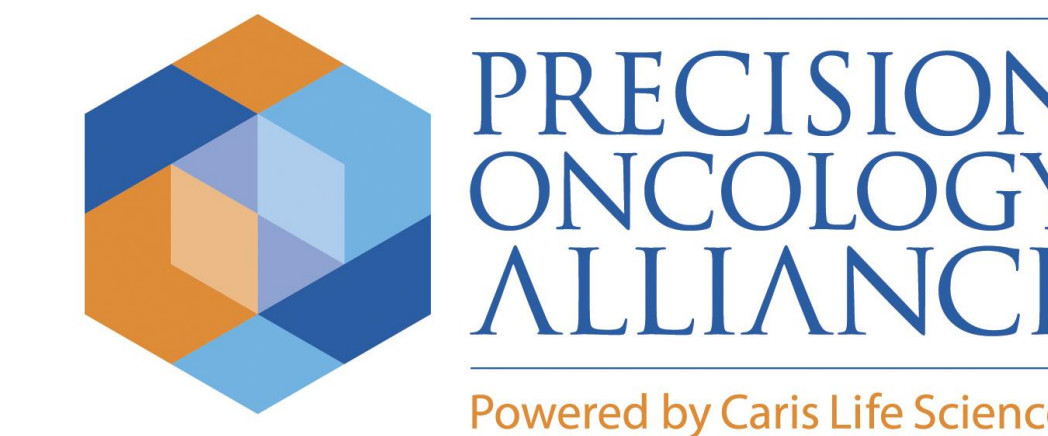




Incidence of *Neuregulin1 (NRG1)* gene fusions across tumor types

¹Stephen V. Liu, ²Rebecca A. Feldman, ³Hossein Borghaei, ⁴Shirish Gadageel, ⁵Patrick C. Ma, ⁶Jorge Nieva, ⁷Alexander Spira, ⁸Ari Vanderwalde, ⁴Antoinette Wozniak, ¹**Sushma Jonna**, ⁹Edward S. Kim
¹Georgetown University, ²Caris Life Sciences, ³Fox Chase Cancer Center, ⁴Karmanos Cancer Institute, ⁵WVU Cancer Institute, West Virginia University, ⁶University of Southern California, Norris Cancer Center, ⁷Virginia Cancer Specialists, ⁸West Cancer Center, ⁹Atrium Healthcare, Levine Cancer Institute
Corresponding author: Stephen V. Liu, stephen.v.liu@gunet.georgetown.edu



Abstract

Background: *NRG1* gene fusions are an emerging potential therapeutic target in non-small cell lung cancer (NSCLC). *NRG1* is a ligand for the HER3 tyrosine kinase and *NRG1* fusions can lead to activation of oncogenic HER2/HER3 and PI3K-AKT signaling.¹ The pan-ErbB inhibitor afatinib has been associated with durable response in patients with *NRG1*+ lung adenocarcinoma.² *NRG1* fusions and the specific fusion partners have not been well characterized across different tumor types.

Methods: Tumor samples submitted for profiling between 01/16 - 04/18 at a CLIA-certified genomics laboratory (Caris Life Sciences, Phoenix, AZ) were assayed with anchored multiplex PCR for targeted RNA sequencing with the ArcherDX fusion assay (Boulder, CO). Novel isoforms and fusions with high reads (defined as >10% of total reads), high confidence after bioinformatics filtering, and considered in-frame, are included in this analysis.

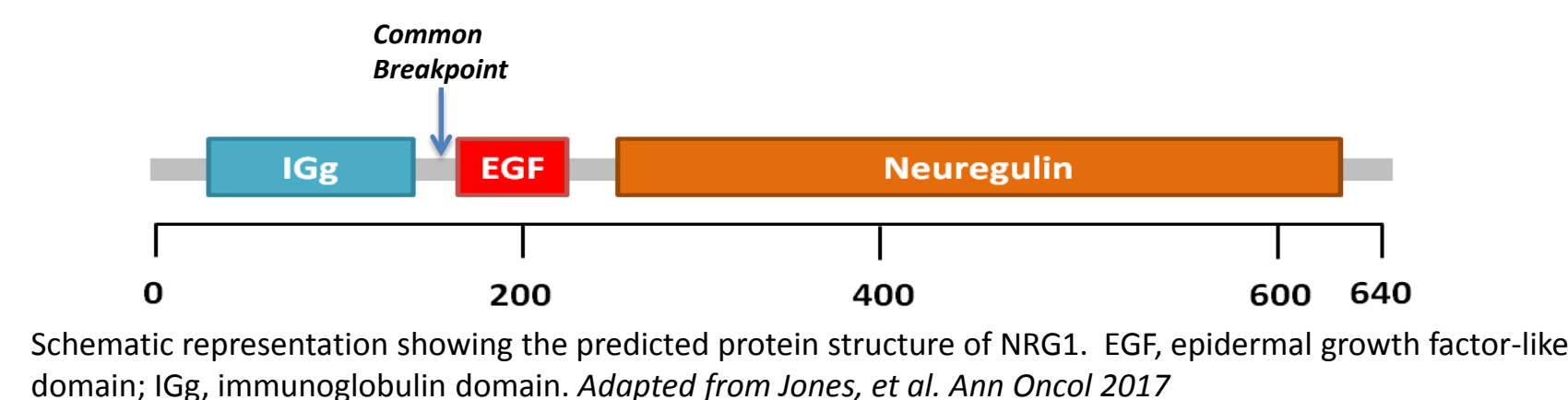
Results: In a cohort of 15,901 tumors successfully assayed, 32 cases (0.2%) harbored an *NRG1* fusion. The incidence of *NRG1* fusions varied by tumor type: 0.8% cholangiocarcinoma (2/257), 0.7% thyroid (1/134), 0.5% ovary (3/589), 0.4% pancreas(2/540), 0.3% NSCLC (20/6648), 0.2% breast (2/962), 0.2% sarcoma (1/498) and 1 case in sinonasal teratocarcinoma (SNTC). One of the 20 NSCLC cases (*NRG1*- SDC4) had squamous histology, the remaining were adenocarcinoma. No *NRG1* fusions were detected in colorectal cancer (0/1456) or glioblastoma multiforme (0/1355). In NSCLC, *NRG1* fusions were mutually exclusive with oncogenic alterations in *EGFR*, *ALK*, *ROS1*, *RET*, and *KRAS* with the exception of one case that co-occurred with a *KRAS* G12C mutation.

Tumor Type	<i>NRG1</i> fusion partners
NSCLC	CD74, n=10; SDC4, n=3; SLC3A2, ATP1B1, TNC, MRPL13, WRN, WDR53, PARP8 (n=1, each)
Ovary	SETD4, TSHZ2, ZMYM2
Pancreas	CDH1, VTCN1
Breast	ADAM9, COX10-AS1
Thyroid	TRAF3IP2
Cholangiocarcinoma	NOTCH2, ATP1B1
Sarcoma	WHSC1L1
SNTC	HMBOX1

Conclusion: Gene fusions in *NRG1* can be identified in various tumor types, though the highest number of events was in NSCLC. Consistent detection of *NRG1* fusions will need to account for multiple fusion partners. The optimal treatment of tumors harboring *NRG1* fusions needs to be established.

Background

- Neurogulin-1 (NRG1)* encodes an EGF-like domain that can serve as a ligand for ERBB3 receptors.^{1,2}
- NRG1* fusions can result in transmembrane proteins that bind ERBB3 and lead to heterodimerization with ERBB2 with subsequent activation of downstream signaling partners including ERK, PI3K, AKT, and NF-κB.¹⁻⁴
- NRG1* fusions have been reported in a variety of tumors and multiple reports have described responses to the irreversible pan-HER inhibitor afatinib and ERBB3 directed therapy.^{2,5-7}
- The incidence of *NRG1* fusions across tumor types is not established.



Methods

Retrospective evaluation of 15,901 tumor specimens tested by next generation sequencing (NGS, Illumina Next Seq, 592 gene panel), select immunohistochemistry (IHC) and fusion analysis at a CLIA-certified lab (Caris Life Sciences, Phoenix, AZ)

RNA-sequencing by ArcherDx fusion assay (FusionPlex solid tumor kit, 53 gene panel) based on anchored multiplex PCR. Only fusions with high reads (> 10% total reads), high confidence after bioinformatics filtering, and considered In-frame were analyzed.

Results

We identified 32 patients whose tumors harbored *NRG1* fusions: NSCLC (n=20), ovarian (n=3), breast (n=2), cholangiocarcinoma (n=2), pancreatic (n=2), sarcoma (n=1), papillary thyroid (n=1) and teratocarcinoma (n=1).

Table 1. Patient Tumor Characteristics

	NSCLC	Ovarian	Breast	Pancreatic/Cholangio	Other
Number	20	3	2	4	3
Median Age (range)	70 (52-90)	57 (47-69)	44 (38-49)	46 (37-48)	65 (36-81)
Sex, n (%)					
Male	8 (40%)	-	-	1 (25%)	2 (67%)
Female	12 (60%)	3 (100%)	2 (100%)	3 (75%)	1 (33%)
Histology, n (%)					
Adenocarcinoma	19 (95%)	-	2 (100%)	4 (100%)	-
Serous carcinoma	-	3 (100%)	-	-	-
Papillary carcinoma	-	-	-	-	1 (33%)
Other	1 (5%)	-	-	-	2 (67%)
Specimen Site, n (%)					
Primary	13 (65%)	2 (67%)	-	-	2 (67%)
Distant Metastasis	7 (35%)	1 (33%)	2 (100%)	4 (100%)	1 (33%)

Results, continued

Figure 1. Incidence of *NRG1* Fusions

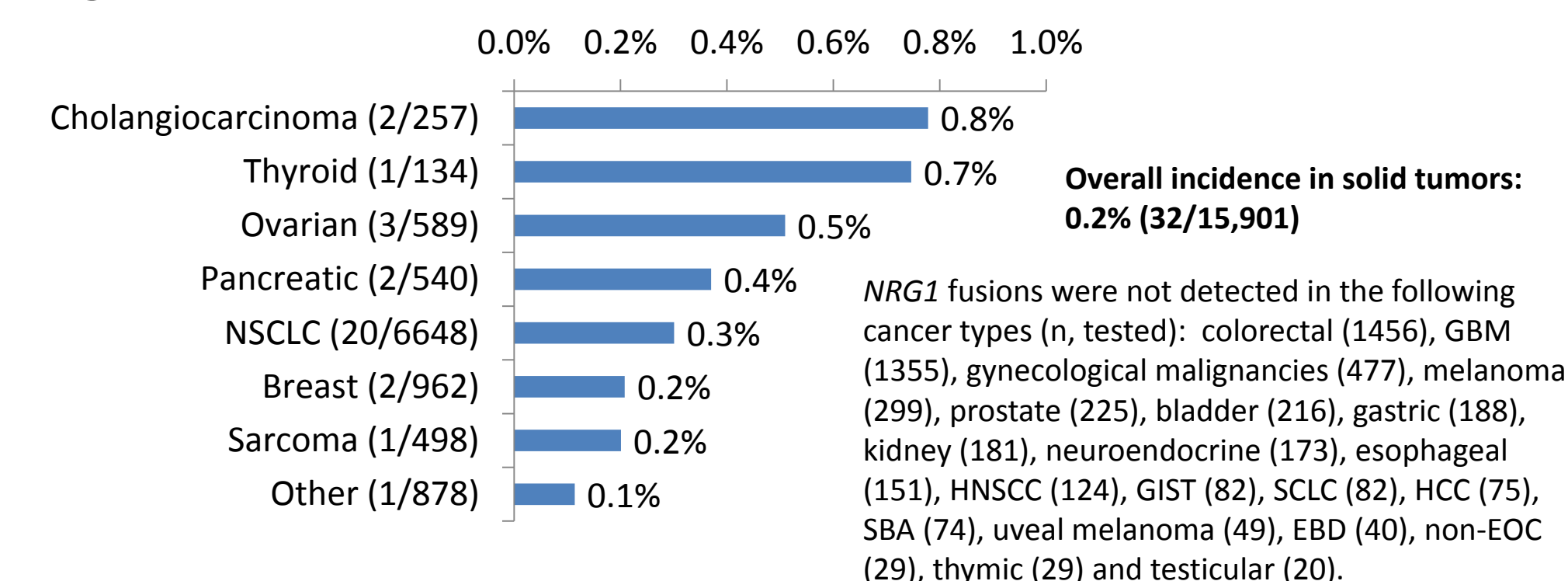


Table 2. *NRG1* Fusions in NSCLC (n=20)

n	Fusion product	Partner: break point	<i>NRG1</i> : break point	Described Previously?
5	CD74: <i>NRG1</i>	CD74: intron 4	exon 5	NSCLC (Fernandez-Cuesta, 2014)
5	SDC4: <i>NRG1</i>	SDC4: exon 2	exon 5	NSCLC (Jones, et al. 2017)
1	SLC3A2: <i>NRG1</i>	SLC3A2: exon 2	exon 2	NSCLC (Gay, et al. 2017)
1	ATP1B1: <i>NRG1</i>	ATP1B1: exon 1	exon 5	Cholangiocarcinoma (Jones, et al. 2017)
1	TNC: <i>NRG1</i>	TNC: exon 10	exon 5	No
1	MRPL13: <i>NRG1</i>	MRPL13: exon 2	exon 2	No
1	WRN: <i>NRG1</i>	WRN: exon 30	exon 2	NSCLC (Dhanasekaran, et al. 2015)
1	WDR53: <i>NRG1</i>	WDR53: exon 2	exon 5	No
1	PARP8: <i>NRG1</i>	PARP8: exon 1	exon 2	No

Table 3. *NRG1* Fusions in other Solid Tumors (n=12)

n	Tumor	Fusion product	Partner: break point	<i>NRG1</i> : break point	Described Previously?
1	Cholangiocarcinoma	NOTCH2: <i>NRG1</i>	NOTCH2: exon 4	exon 5	Cholangiocarcinoma (Jones, et al. 2017)
1		ATP1B1: <i>NRG1</i>	ATP1B1: exon 2	exon 2	
1	Pancreas	CDH1: <i>NRG1</i>	CDH1: exon 2	exon2	No
1		VTCN1: <i>NRG1</i>	VTCN1: exon 2	intron 3	
1	Ovarian	SETD4: <i>NRG1</i>	SETD4: exon 2	exon 2	No
1		TSHZ2: <i>NRG1</i>	TSHZ2: exon 1	exon 5	
1	Breast	ZMYM2: <i>NRG1</i>	ZMYM2: exon 2	exon 2	No
1		ADAM9: <i>NRG1</i>	ADAM9: intron 16	exon 2	
1	Papillary Thyroid	COX10-AS1: <i>NRG1</i>	COX10-AS1: exon 1	exon 2	No
1	Sarcoma, NOS	TRAF3IP2: <i>NRG1</i>	TRAF3IP2: exon 9	intron 3	No
1		WHSC1L1: <i>NRG1</i>	WHSC1L1: exon 1	exon 2	
1	Teratocarcinoma	HMBOX1: <i>NRG1</i>	HMBOX: exon 1	exon 5	No

Results, continued

Table 4. Co-occurring mutations in <i>NRG1</i> fusion patients						
	Biomarker	Lung	Ovarian	Cholangio	Pancreatic	Breast
Fusion	ALK	0% (0/16)	-	-	-	-
	ROS	0% (0/16)	-	-	-	-
NGS	EGFR	0% (0/16)	0/3	0/3	0/2	0/2
	KRAS	6% (1/16)	0/3	0/3	0/2	0/2
	PTEN	0% (0/16)	1/3	0/3	0/2	0/2
	AKT	0% (0/16)	0/3	0/3	0/2	0/2
	PIK3CA	0% (0/16)	1/3	0/3	0/2	0/2
	TP53	50% (8/16)	3/3	0/3	0/2	1/2
	TMB_high*	12.5% (2/16)	0/3	0/3	0/2	0/2
	ERBB2	0% (0/16)	0/3	0/3	0/2	0/2
	ERBB3	0% (0/16)	0/3	0/3	0/2	0/2
	ERBB4	0% (0/16)	0/3	0/3	0/2	0/2
IHC	PTEN_loss**	12.5% (2/16)	1/2	-	-	1/2
	PD-L1 (22c3)†	33% (6/18)	-	-	-	-
	PD-L1 (SP142)‡	-	0/3	0/2	0/2	0/2
	HER2	-	-	-	-	0/2
	HR (ER/PR)	-	-	-	-	1/2

*TMB, tumor mutation burden high, ≥17 mutations/Mb, **PTEN loss =0+100%
†PD-L1 (22c3) for 6 cases, 1+ 50%, 2+15%, 1+5%, 2+1%, 1+ 60%, 1+ 40%, ‡PD-L1 (sp142) positivity is ≥2+ and ≥5%

Conclusions

- NRG1* rearrangements represent an emerging molecular subtype of solid organ malignancies.
- These chimeric fusions are most commonly detected in NSCLC, but are also identified in various other tumor types.
- A diversity of fusion partners to *NRG1* were detected in our study, including novel fusion variants not previously described. The significance of each fusion partner is not yet known.
- Further prospective studies are needed to establish the role of targeted therapy for patients with tumors harboring *NRG1* fusions.

References

- Fernandez-Cuesta L, Plenker D, Osada H, et al. CD74-*NRG1* fusions in lung adenocarcinoma. *Cancer Discov* 2014.
- Drilon A, Somwar R, Mangatt BP, et al. Response to ERBB3-directed targeted therapy in *NRG1*-rearranged cancers. *Cancer Discov* 2018.
- Trombetta D, Graziano P, Scarpa A, et al. Frequent *NRG1* fusions in Caucasian pulmonary mucinous adenocarcinoma predicted by Phospho-ErbB3 expression. *Oncotarget* 2018.
- Murayama T, Nakaoku T, Enari M, et al. Oncogenic fusion gene CD74-*NRG1* confers cancer stem cell-like properties in lung cancer through a IGF2 autocrine/paracrine circuit. *Cancer Res* 2016.
- Gay ND, Wang Y, Beadling C, et al. Durable response to afatinib in lung adenocarcinoma harboring *NRG1* gene fusions. *J Thorac Oncol* 2017.
- Jones MR, Lim H, Shen Y, et al. Successful targeting of the *NRG1* pathway indicates novel treatment strategy for metastatic cancer. *Ann Oncol* 2017.
- Cheema PK, Doherty M, Tsao MS. A case of invasive mucinous pulmonary adenocarcinoma with a CD74-*NRG1* fusion protein targeted with afatinib. *J Thorac Oncol* 2017.
- Dhanasekaran SM, Balbin OA, Chen G, et al. Transcriptome meta-analysis of lung cancer reveals recurrent aberrations in *NRG1* and Hippo pathway genes. *Nat Commun* 2014.