Review Article

NRG1 fusions in non-small cell lung cancer: a narrative review on biology, detection and therapy

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Background and Objective: Neuregulin 1 (NRG1) is a small ligand of the tyrosine kinase receptors (TKRs) that was recently described as a new molecular trait of non-small cell lung cancer (NSCLC). *NRG1* gene rearrangements mainly occur in the rare NSCLC subtype of lung invasive mucinous adenocarcinomas (IMAs), but are also reported with low frequency in non-IMA NSCLC and many other solid tumors. The NRG1 oncogenic fusions enhance the ectopic expression of the NRG1/ErbB3 receptor-ligand complex, thus inducing the activation of PIK3CA-AKT/MAPK pathways. The most recent scientific advances highlight the potential use of the NRG1 fusions as agnostic biomarkers and add support to the key role of NRG1/ErbB3 axis deregulation in NSCLC cancer onset and evolution. The aim of this narrative review was to give a concise overview of the current knowledge on the biology and the predictive/prognostic role of the NRG1 fusions, as well as to summarize and update the available detection methods and pharmacological approaches related to their role as prognostic and predictive marker in NSCLC.

Methods: The main available scientific advances about NRG1 fusions in NSCLC published from 1992 until April 2023 were summarized starting from the main published data in English language on international peer-reviewed, high-quality journals and official sites. Data about clinical trials were from official sites.

Key Content and Findings: The main knowledge about the biological role of NRG1 fusions, as well as the impact of co-occurrence with other NSCLC driver genes, strengths and limitations of different proposed workflows to detect NRG1 fusions were summarized. The clinical value of NRG1 fusions in tyrosine kinase inhibitor (TKI) treated patients along with the main results from the ongoing clinical trials and drug discovery in NSCLC updated to April 2023, extended to other solid tumors, were also included.

Conclusions: This review supports the role of NRG1 fusions as promising molecular marker for therapy decision and monitoring NSCLC patients, but also corroborate the agnostic role of these fusions among solid tumors. Further research is required to clarify the structure, the function and the oncogenic aggressiveness of the NRG1 fusions in carrier patients and expand the precision medicine portfolio.

Keywords: Neuregulin 1 (NRG1); gene fusion; non-small cell lung cancer (NSCLC); target therapy

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Introduction

Background

Human neuregulins (NRGs) are small ligands belonging to the epidermal growth factor (EGF) family proteins. They are encoded by four different genes (*NRG1-4*) and are implicated in the activation of ErbBs receptors to modulate both physiologic and neoplastic processes (1). Globally, many different NRG1 fusion variants, related to having an ErbB3-related oncogenic activity, were identified to date in many solid tumors, used non-homogeneous methodological approaches (2).

Rationale and knowledge gap

Fusions genes represent the most promising and targetable genetic aberrations in cancer patients. Among these, NRG1 fusions are one of the most investigated markers in the latest years, due to its agnostic features and the observed clinical relevance highlighted in many scientific contexts. Even uncommon, the NRG1 fusions were reported in a clinically relevant portion of patients with non-small cell lung cancer (NSCLC) and are one of the distinctive molecular feature of lung invasive mucinous adenocarcinomas (IMAs) subtype, that showed the highest rate of NRG1 fusions described to date. For this reason, lung cancer is actually one of the most interesting model to sieve *NRG*-rearranged genomic features, the heterogeneity of fusion variants, and their role in tumor biology (2,3). More recently, the NRG1 fusions were listed among the molecular lesions associated to innate and acquired resistance to tyrosine kinase therapies in oncogene-addicted NSCLCs, thus arousing a recent and ever increasing interest in these groups of lung cancer patients.

However, many details about how the activation of the NRG1/ErbB signal due to NRG1 fusions may influence lung adenocarcinomas progression during targeted therapy with tyrosine kinase inhibitors (TKIs) remain uncovered (4,5). Due to the actual poor knowledge of the biological consequences of many discovered NRG1 fusion variants, the utility of tracking this group of lesions in patients under standard chemotherapy, as well as immunotherapy and targeted therapies are encouraging, but not jet fully agreed. Pharmacological inhibition of NRG1/ErbB ligation or ErbBs receptor dimerization and activation was observed using different agents with mixed success (5).

Objective

Since its first identification, research studies on NRG1 fusions in NSCLC are constantly increasing, since they actually represent one of the most promising markers for this group of lung tumors. Basic science evidences, as well as the technical workflow aimed to investigate these complex rearrangements are often fragmented and need continuous updating.

The aim of this review is to summarize the actual knowledge of the biological role, the detection methods and the translational power of NRG1 fusions, in order to allow a rapid overview of this field to biologists and clinicians. Scientific advances in technical approaches used to identify NRG1 fusions, the biological contribution of such fusions and the latest translational evidences are updated to year 2023, giving the most recent overview in this field to readers. All referred data are according to Table S1. I present this article in accordance with the Narrative Review reporting checklist (available at https://pcm.amegroups.com/article/view/10.21037/pcm-23-2/rc).

Methods

The author compiled a narrative overview of the main available scientific knowledge about NRG1 fusions, which was published in English language from 1992 and updated until April 2023 by international peer-reviewed journals, available on PubMed. Scientific data were used to summarize the recent advances in the biological know-how about NRG1 and technological approaches to identify NRG1 fusions in NSCLC and other solid tumors. The main keywords searched were: "NRG1", "ErbB3 AND NRG1", "lung cancer AND NRG1", "NRG1 fusion", "NRG1 rearrangement" "invasive mucinous adenocarcinoma AND NRG1 fusion", "NRG1 AND cancer", "NRG1 fusion detection", "TKI AND NRG1", as detailed in Table S1. Results from large studies were privileged over case reports, which are only referenced in key contexts lacking more documentary evidence. Updates on preclinical and clinical advances were also from official clinical sites (https://clinicaltrials.gov/).

Discussion

The biological role of NRG1 fusions in tumors

The NRG1 belongs to the epidermal growth factor (EGF) family pleiotropic ligands and it is encoded by the homonymous *NRG1* gene, which is located at chromosome 8p12 (5). It is the best characterized member within the

family of neuregulins and is mainly expressed by healthy cells of neural and non-neural origin to mediate cell-cell interactions, including epithelium, nerve, cardiac and skeletal muscles. Alternative promoters activation and events of alternative exon splicing generate many NRG1 isoforms (6-8), which can be distinguished based on differences in their NH2-terminal regions as follows: type I-NRG1 (NDF, HRGs, ARIA), type II-NRG1 (GGFs) and type III-NRG1 (SMDF) (9). All bioactive NRG1 isoforms exert their ligand function by linking the extracellular portion of ErbB receptors through the EGF-like domain, which represents the main shared feature of neuregulins. The NRG1 binds and activates via paracrine or autocrine signaling the ErbB receptors located on the cell surface (mainly ErbB2 and ErbB3) with different affinity, depending on the isoform type (α- or β-isoforms) to finally modulate proliferation, survival, migration, and differentiation events in many cellular compartments (10). The activation of both ErbB2 and ErbB3 requires a heterodimerization process to initiate the signaling cascades mediated by phosphoinositide 3-kinases/serine threonine protein kinase (PIK3/AKT) and mitogen activated protein kinase (MAPK), because ErbB2 is an orphan receptor, whereas and ErbB3 has weak kinase activity (10-12).

Aberrant chimeric NRG1 ligands are transversally reported in solid tumors to retain the active EGFR-like domain by which they can abnormally and ectopically bind and activate ErbBs and impact on specific cellular pathways through multiple mechanisms (5). Considering the different affinity of neuregulins for ErbB receptors, each fusion variant of NRG1 might have multiple effects and different clinical significance that need to be functionally investigated (5). The most frequent fusion variants of NRG1 reported to date are the cluster of differentiation 74 (CD74)/NRG1 and solute carrier family 3 member 2 (SLC3A2)/NRG1 fusions, that lead the overexpression in lung tissue of the neuronal NRG1III-β3 isoform, which in turn swicth off the ErbB2/ErbB3 heterodimerization process (11,12). In lung cancer, the SLC3A2/NRG1 fusion also plays an essential role in cancer cell proliferation and tumor growth via the PIK3/extracellular signal-regulated kinase (ERK)/mammalian-mechanistic target of rapamycin (mTOR) pathway (13,14).

The molecular and pathological features of NRG1-positive tumors have taken shape over the past two years. NRG1 fusions are more common in patients with no smoking history, are predominantly associated with tumors having an adenocarcinoma histology, both in primary and metastatic sites, and are strictly related to an aberrant ErbB3 phosphorylation process. In lung cancer, they are particularly enriched in the IMA subtype (7–31%), a rare malignance and aggressive subtype of adenocarcinoma (5,15). In lung IMA, the NRG1 fusions are reported in metachronous nodules, thus supporting the clonal nature of these molecular lesions (16). Through rare (~0.2%), the NRG1 fusions are also found across more than 10 solid tumor types, including pancreatic (up to 6% of ductal adenocarcinoma subtype), gallbladder and bile duct cancers, ovarian and sarcoma cancers, head and neck cancers, breast, kidney, prostate, colorectal and bladder tumors (2,3,17,18).

Looking at the molecular background of patients, the NRG1 fusions frequently occur in tumors without any other gene-driver lesions (3), even if they are occasionally reported to be associated to other gene fusions in naive tumors and/or NSCLC re-biopsies (2,19,20). By contrast, recent data from studies on single tumor cohorts as well as from the global and international registry of NRG1 fusions seem to confirm the co-occurrence status of kirsten rat sarcoma virus (*KRAS*) mutations. The main hypothesis is that KRAS p.Gly12Cys mutant protein, but also other mutations in the hot spot regions of *KRAS* gene could exert a synergic effect in activating ErbB pathway and malignant NSCLC phenotype maintenance through the activation of SLC3A2-NRG1 chimera by ADAM metallopeptidase domain 17 (ADAM17), thereby enhancing the RAS and ErbBs signalling, thus increase proliferation by activating the EGFR-ERK signalling (2,4). Anyway, more confirmative studies on different NRG1 fusion variants and in larger cohorts are demanded to corroborate this fascinating data.

The correlation among NRG1 fusions, programmed cell death ligand 1 (PD-L1) and tumor mutation burden (TMB) status was also recently discussed. The NRG1 fusions clustered in NSCLC with low TMB, whereas it still needs to be clarified its correlation with microsatellite instability in lung IMA. Data on immune-checkpoint proteins linkage are not conclusive in lung IMA, where the PD-L1 protein appears typically absent or low present. Anyhow, the NRG1 fusions appear rarely linked to PD-L1 expression (4%), as reported by the eNRGy1 Global Multicenter Registry (21). The correlation of NRG1 fusions with other immuno-checkpoints is totally missing.

The detection of NRG1 fusions

The oncogenic role of *NRG1* gene rearrangements in solid tumors dates back to the first observation made by Liu *et al.* in 1999 on breast cancer cell lines (22), and it was

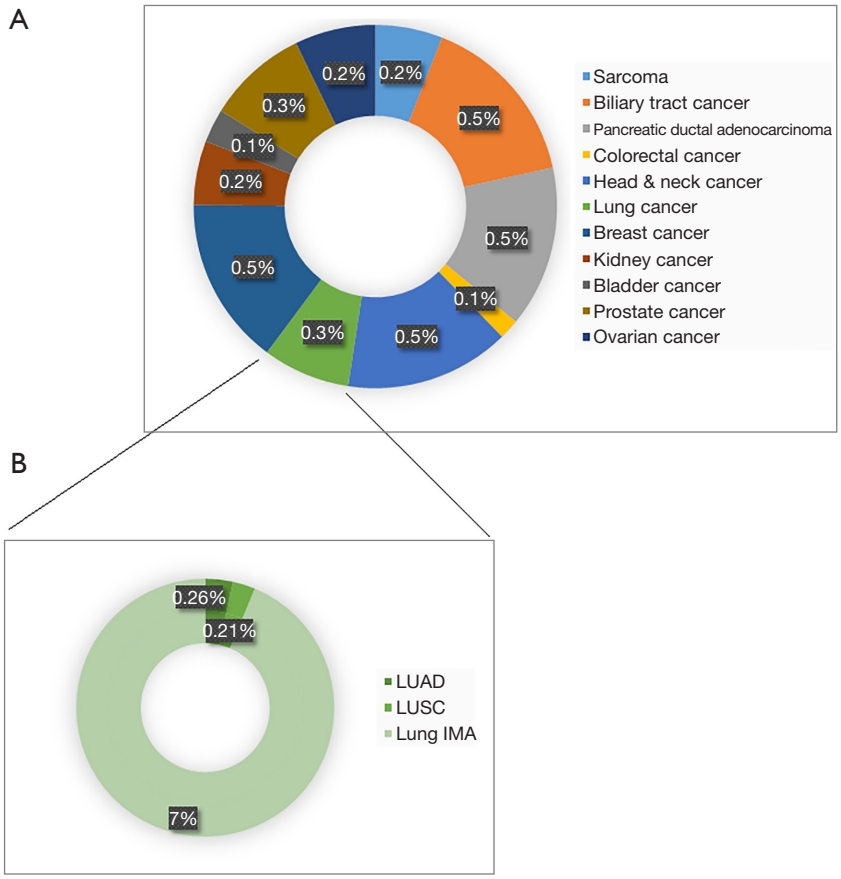

Figure 1 Rate of NRG1 fusions by tumor type. (A) The values are only referred to screening by transcript detection in tumors not sorted by other driver co-occurrent mutations (3,24). (B) Rate of NRG1 fusions in LUAD, LUSC and lung IMA. The values are only referred to screening by transcript detection (11,15). LUAD, lung adenocarcinoma; LUSC, lung squamous adenocarcinoma; IMA, invasive mucinous adenocarcinoma.

then reported in 2003 in pancreatic cancer cell lines (23). Already at the time, several breakpoints at *NRG1* chromosomal region using fluorescent in situ hybridization (FISH) were found having an extensive complexity in terms of chimeric gene structure (23). The translational impact of this finding emerged only in 2014, thanks to the first report of the CD74-NRG1βIII fusion by Fernandez-Cuesta *et al.* in five lung IMAs from female Asiatic patients (11). NRG1 fusions rarely occur in a wide range of cancer types, with an observed global incidence of 0.2% (3,24). Most of the NRG1 fusion variants having oncogenic activity were reported in NSCLC patients, mainly linked to adencarcinoma histology, even if the incidence are low also in this type of tumor (0.3%, *Figure 1*) (3,5,17,24,25).

The *CD74* is the most frequent among the NRG1 fusion partner genes, but several other partners were reported across tumors. In NSCLC, less frequent fusion partners include the Syndecan-4 (*SDC4*), *SLC3A2*, RNA-binding protein with multiple splicing (*RBPMS*), Werner (*WRN*), vesicle associated membrane protein 2 (*VAMP2*), Kinesin Family Member 3B (*KIFI3B*), THAP Domain Containing 7 (*THAP7*), SMAD Family Member 4 (*SMAD4*), ATPase Na^+/K^+ Transporting Subunit Beta 1 (*ATP1B1*), Tenascin C (*TNC*), Midkine (*MDK*), Mitochondrial Ribosomal Protein L13 (*MRPL13*), Disco Interacting Protein 2 Homolog B (*DIP2B*), Rho Associated Coiled-Coil Containing Protein Kinase 1 (*ROCK1*), Poly (ADP-Ribose) Polymerase Family Member 8 (*PARP8*), Dihydropyrimidinase Like 2 (*DPYSL2*), Integrin Subunit Beta 1 (*ITGB1*), Dedicator Of Cytokinesis 5-GeneCards (*DPCK5*), LMBR1 Domain Containing 1 (*LMBRD1*), WD Repeat Domain 53 (*WDR53*), ATP synthase subunit beta (*ATP5B*), Tetratricopeptide Repeat,

Table 1 Strengths and limitations of the main RNA-based technologies used to detect NRG1 fusions in NSCLC and other solid tumors

Technology	Strengths	Limitations	References
WTS	Can detect NRG1 fusions, even if the partner gene is unknown	High-quality RNA is required	(11,15,27-38)
AMP-NGS	Can detect multiple NRG1 fusions, even if the partner gene is unknown	Detection is limited to the region of mapped primers	(2,3,15,16,18,27,39-41)
RNA-targeted NGS	Can capture many fusion variants, allowing identification of in-frame transcripts even in genes with multiple splice variants and transcriptional start sites	Rare fusion variants not included in the panels cannot be detected	(17,19,20,25,27,41-50)
Nanostring Technology	Can efficiently estimate the level of expression of all the exons in the gene of interest	The assay may not be easily optimized if the quality of the RNA is low	(2,27,51-53)
	Can detect complex rearrangements	Unknown 5' fusion partners cannot be identified	
RT-PCR	Efficient to detect the 5' and 3' partner genes if breakpoints are known and highly recurrent	3'–5' imbalance is not reliable to detect NRG1 fusions	(2,11,40,53-57)
		Can detect a limiting number of NRG1 fusion variants	
		More rapid and less expensive than NGS	

NSCLC, non-small cell lung cancer; WTS, whole transcriptome sequencing; AMP-NGS, anchored multiplex PCR-next generation sequencing; RT-PCR, real-time polymerase chain reaction.

Ankyrin Repeat And Coiled-Coil Containing 1 (*TANC1*), Syndecan Binding Protein (SDCBP), RAB11 Family Interacting Protein 1 (*RAB11FIP1*), MOK Protein Kinase (*PMOK*), Mitochondrial Ribosomal Protein L3 (*MRPL13*), LMBR1 Domain Containing 1 (*LMBRD1*), EFR3 Homolog A (*EFR3A*) and F11 Receptor (*F11R*) genes (26).

Both the rarity of the NRG1 fusions and the diversity of gene fusion partners make detecting these types of lesions very challenging. One of the main difficulties remains to define a robust diagnostic tool to capture all possible NRG1 fusions in the available biological samples of patients using a harmonic, and good cost-effective screening strategy. The published papers reported in fact a variable range of *NRG1* rearrangements in lung IMA as well as in many other solid tumors, frequently due to the non-homogeneous screening approaches used and the complex nature of these chromosomal rearrangements that make difficult their detection (2,5). Moreover, a small number of NRG1 fusion variants are included in few commercially available panels for next generation sequencing (NGS) and this, in turn, penalizes the identification and discrimination of the NRG1 oncogenic fusions from the non-oncogenic ones, both in NSCLC and other tumors (*Tables 1*,*2*).

To identify *NRG1* fusions, the most valuable and actually suggested approach remains to perform a pre-screening to identify phosphorylated ErbB3 (pErbB3) expression in tumor tissues using immunohistochemistry (IHC), followed by NGS (5,26). A very good correspondence between tumor samples expressing pErbB3 and the presence of the NRG1 oncogenic fusions corroborating the utility of this methodological indication, as well as the previously published data on the acceptable stability of pErbB3 in the normal routine of histopathological analysis (2,60). The available data support the idea that the time to fixation remains one of the most critical factors in preserving phosphorylated proteins in tissues. Moreover, a heterogeneous staining pattern from perimeter to center can be observed on the whole sections due to the instability of phosphorylated protein. As a consequence, the high widely reported match between pErbB3 expression and NRG1 oncogenic fusions highlights the importance of preservation of phosphorylated proteins in tissues by conventional fixatives.

Among the NGS approaches, the RNA-based sequencing clearly offers the advantage to identify in-frame oncogenic fusions and allows the discrimination of transcribed

Table 2 Strengths and limitations of the main DNA-based and indirect technologies used to detect NRG1 fusions in NSCLC and other solid tumors

Technology	Nucleic acid/protein	Strengths	Limitations	References
WES	DNA	Querying multiple NRG1 fusions at the same time	No information about the transcription of rearranged *NRG1* gene is obtained	(24,28,29,31,38,54)
		Can establish the exact breakpoint and frame of quite all NRG1 fusions variants	Does not cover large intronic regions. Failure in detection of unusual intronic or unknown breakpoints	
DNA-targeted NGS	DNA	Querying multiple NRG1 fusions at the same time	Genomic coverage may not be comprehensive. Sensitivity for fusion detection can be reduced if intronic sequences are large and difficult to completely cover	(2,16,24)
		Can establish the exact breakpoint and frame only of targeted NRG1 fusion variants	No information about transcription of rearranged *NRG1* gene is obtained	
			Does not cover large intronic regions. Failure in the detection of unusual or unknown breakpoint/gene partner in unknown *NRG1* fusion variants having unknown breakpoints	
Sanger sequencing	DNA	Useful to sequence the exact genomic breakpoints in cases of well known NRG1 fusion variants	Low sensitivity, biological material and time consuming	(46,57,58)
FISH	DNA	Pericentric and paracentric fusions, fusions could be detected (break-apart probes preferred)	Does not give indication about the presence and sequence of *NRG1* fusion transcripts	(2,20,24,25,32,40,45,59,60)
		Fusion gene partners could be detected using specific probe (dual fusion assays)	Is not optimal in a context of multiplex screening	
IHC (pErbB3)	Protein	Can measure the active (phosphorylate) state of ErbB3 receptor	Variable sensitivity and specificity, are also related to the tissue block management. ErbB3 expression could be elevated also in the absence of *NRG1* fusions	(20,38,55,56,60)
		Gives indication about the potential oncogenicity of NRG1 fusions and could be used as pre-screening methods in case of low material or large screening cohorts	No gene fusion partner indication	

NSCLC, non-small cell lung cancer; WES, whole exome sequencing; NGS, next-generation sequencing; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry.

products from alternative splicing events, as happens for neuregulins family genes. Actually, there are no available commercial amplicon-based or hybrid-capture targeted-NGS panel able to cover all NRG1 fusion variants; so, the most complete approaches to identify all possible in-frame NRG1 transcripts remain the whole transcriptome sequencing (WTS) or the anchored multiplex polymerase chain reaction (PCR) (AMP)-RNA sequencing (*Table 1*). Considering the high number of NRG1 transcript fusion variants reported in a few years and the importance of discriminating those having an oncogenic activity, the additional reported RNA-based molecular approaches, such as 3'/5' expression ratio calculation by Nanostring technology (31,61) or real-time (RT) PCR actually remain

underused and very limiting.

All the available DNA-based testing methods, such as DNA-based NGS by whole exome/genome sequencing (WES/WGS), offer the advantage to provide the exact sequence of the genomic breakpoints, but have the great limitation to provide the only evidence of an existing chromosomal rearrangement and cannot capture large and difficult intronic regions to sequencing (present in the *NRG1* gene) or discriminate in-frame NRG1 transcripts (*Table 2*).

The FISH was investigated in few papers as a possible *in situ* screening method to detect *NRG1* rearrangements. Anyhow, the real utility of this approach has not been clarified yet, since it remains indaginuous and tissue comsuming and still lacks a well-established and fully validated cut-off of positivity (59,60). Moreover, similarly to the other just reported ALK and ROS1 fusions in lung cancer, the FISH patterns of *NRG1* break showed both split and isolated 3' signals having a not jet established value (60). Finally, the few available data comparing FISH and NGS results obtained by RNA-based AMP-NGS technology revealed that not all NRG1-positive FISH cases have fusion transcripts (62), thus confirming the idea that, at this moment, the FISH cannot be included in the diagnostic workflow to detect NRG1 fusions.

Finally, liquid biopsy was recently discussed as an alternative and non-invasive way to identify the NRG1 fusions both for diagnostic and disease monitoring contexts. Taking into account the just available technical approaches to detect gene fusions in tumor tissues having high sensitivity and specificity, it will become intriguing to assess if these quality parameters are also acceptable in a liquid biopsy setting to detect NRG1 fusions for those patients whose tissues are not available. Actually, there are no published data about this.

Clinical actionability of NRG1 fusions

The NRG1 fusions are linked to the aberrant tyrosine kinase activity of ErbB2/ErbB3 heterodimers with consequent activation of PI3K-AKT and the MAPK pathways, thus falling into the amazing bridge between the ErbB network and targeted approved/in development anticancer therapies (1).

To date, the presence of NRG1 fusions in NSCLC patients and other tumors has been correlated with worse overall survival (OS) and disease-free survival under current therapies regimen. The NRG1 fusions were listed among molecular markers for poor prognosis in patients treated with standard chemotherapy, chemoimmunotherapy or immune checkpoint inhibitors, thus corroborating the idea that current approaches should be re-evaluated and/or revised for this specific group of patients (2,63).

ErbBs activity inhibition through the interference with the NRG1 lingand-ErbB receptor binding and/or ErbB dimerization process are actually proposed as the best approach to elicit a good clinical response in NRG1-positive patients (63). Small series of NRG1 fusion-driven cancers, including NSCLC, were recently reported to be treated with afatinib, a pan-ErbB family inhibitor, authorized for advanced NSCLCs (27,42,64). Encouraging, but heterogeneous responses were observed in NRG1-positive enrolled NSCLC, listed in the international eNRGy1 Registry and treated with afatinib (2). Among these patients, 5/20 (25%) achieved a partial response, whereas a stable disease was observed in 15% (3/20) of patients. The duration benefits of afatinib in monotherapy were limited, with a progression-free survival (PFS) of 2.8 months, whereas no differences were observed in OS of patients who received afatinib compared to those patients who did not receive afatinib. Finally, most of the patients (60%) showed a progressive disease. NRG1-positive patients with advanced cancer are also actually under enrollment in the Targeted Agent and Profiling Utilization Registry (TAPUR) Study for treatment with afatinib (NRG1-Group 18, last update on April 3, 2023; NCT02693535).

Tarloxotinib, (targeting low oxygen) is a pan-ErbB inhibitor whose activation is due to the low level of oxygen commonly observed in tumors (65). Under hypoxia condition, tarloxotinib pairs a potent kinase inhibitor by the membrane reductase STEP4 protein, a membrane reductase, to generate in the hypoxic tumor environment high levels of tarloxotinib-E. This robust TKI inhibits ErbBs activation by blocking their phosphorylation process and induces tumor regression or growth inhibition, as shown in patient-derived cell lines and multiple murine xenograft models having NRG1 fusion (66). The only related trial was closed without any published results and actually there are no additional open clinical trials with tarloxotinib in NRG1-positive patients (NCT03805841, completed; last update posted on November 19, 2021; no Study Results Posted on ClinicalTrials.gov).

GSK2849330 is a monoclonal antibody that binds the extracellular domain of ErbB3 and blocks the interaction with NRG1 ligand. In the Phase I study NCT01966445, the GSK2849330 showed good safety in patients and evidence of target engagement were observed with no

dose-limiting toxicities. A durable partial response of 19 months using this drug was only observed in the NRG1-positive patient having a CD74-NRG1 fusion, whereas results on patients having a variety of ErbB3/NRG1-expressing advanced tumors were seen to be insufficient to achieve a reliable benefit from treatment with this antibody. Only one patient (3%) overexpressing ErbB3 had a partial response, 7 (24%) had stable disease, 1 had noncomplete response/nonprogressive disease, whereas 16 patients (55%) had progressive disease (NCT01966445, completed, Last Update Posted on July 1, 2019), (56).

Seribantumab (also named MM-121/SAR256212), is a competitor of NRG1 binding with ErbB3, thus blocking the ErbB activation process and downstream cellular pathways activation. The antitumoral efficacy of seribantumab in blocking activation of the four ErbB family members and of downstream signaling was first observed in preclinical models of patient-derived lung and breast cancer cell lines and patients-derived xenograft (PDX) models from lung and ovarian patients having NRG1 fusions, and then confirmed by the encouraging results in randomized Phase II trials enrolling metastatic cancer patients having high NRG1 and/or low ErbB2 expression levels (NCT01447706, NCT01151046 and NCT00994123) (67). The first published findings from the phase 2 CRESTONE (Clinical study of REsponse to Seribantumab in TumOrs with NRG1 fusions) trial (NCT04383210, Recruiting, Last Update Posted on February 14, 2023) were presented at the 2022 American Society of Clinical Oncology (ASCO) Annual Meeting from a limited number of patients. A tumor reduction from baseline with acceptable tolerability was observed in 92% of patients and an encouraging overall response rate of 33% (4/12) in adult patients with metastatic or locally advanced solid tumors harboring NRG1 fusions and 36% (4/11) in those patients having NSCLC was achieved. A complete response was observed in 17% of patients and a partial response rate in 17% too. Fifty-eight percent of patients achieved stable disease, and 8% experienced disease progression. The most durable response was observed in two patients under seribantumab treatment who showed a duration of response of >16 and 11 months (68). The updated efficacy of seribantumab across different tumor types was presented at the 2023 American Association Cancer Research Annual Meeting (Session: Phase II Clinical Trials 2, Abstract Number: CT229). In the cohort of patients with solid tumors harboring NRG1 fusions who received at least one prior therapy and were naïve to ErbB-targeted therapy (Cohort 1), 9% had confirmed complete response, 27% had confirmed partial response, and 59% had stable disease. The overall duration of response ranged from 1.4 to 17.2 months. In NSCLC the overall response rate was 39% and the disease control rate was 94%.

Zenocutuzumab (MCLA-128) is a bispecific antibody which blocks both ErbB2 and ErbB3, thus interfering with NRG1/ErbB3 binding and thereby impacts on downstream ErbB3-related pathways. In 2021 it granted the U.S. Food and Drug Administration Fast Track designation for tumors harboring an NRG1 fusion after failure of standard therapy and it is actually considered the most promising new inhibitors to use in NRG1-positive patients (69). Zenocutuzumab is now under evaluation in the phase 2 of the eNRGy study and early access program (EAP), recruiting patients with locally-advanced unresectable or metastatic solid tumor having a documented *NRG1* gene fusion, identified by PCR, NGS or FISH cancer patients having NRG1 fusions from North America, Europe, and Asia. The first published results demonstrated a robust and durable efficacy regardless of tumor histology. In the cohort enrolled as for January 2022 (85 eNRGy, 14 EAP), the duration of response of 6 months was observed in 70% of patients, with a rare grade ≥ adverse events (NCT02912949, Recruiting, Last Update Posted on December 27, 2022) (70).

New agents against ErbB3 activity are recently proposed as targeted options for NRG1-positive patients (5,38). Among these, anti-drug conjugates targeted ErbB3 showed a potent anti-tumoral effect in many drug resistance contexts of solid tumors, so they could represent an alternative and intriguing approach to block the spreading of tumors overexpressing ErbB3 (5,71).

A good, but variable response to different inhibitors is expected, depending on common/specific oncogenic features of each NRG1 fusion variant, as happens in lung cancer for many well-known driver fusions.

NRG1 fusions/TKI-treatment interface highlights

The ErbB3 activation has been related to drug resistance in many cancer models, as well as its activity was reported as a key factor in drug tolerance of cancer cells. A link between ErbB3 aberrant activation and intrinsic/acquired resistance to the third generation EGFR-TKI osimertinib mediated by the anexelekto (AXL) pathway was just reported in *EGFR* mutant lung cancer cells (72).

The NRG1 fusions were more recently listed among molecular markers of resistance and progression to TKI therapy of NSCLCs with druggable *EGFR* mutations and

gene fusions (5). Ever increasing *in vivo* and *in vitro* evidences were provided in support to this hypothesis. Preclinical study showed that alectinib-resistant cells lost the EML4-ALK driver oncogene, and activated the NRG1/ErbB3 pathway to maintain cell survival alternatively in ALK-rearranged cancer cells with mesenchymal features (73,74).

By using primary cancer cell cultures from pleural effusion of an ALK-positive lung cancer patient, an increase of the NRG1 ligand levels with a consequent activation of ErbB3 pathway has also been seen to be directly related to resistance to crizotinib treatment. The use of the pan-ErbB inhibitor afatinib on lung cancer resistant cells overexpressing NRG1 was able to restore drug sensitivity (75,76). Th use of pan-ErbB inhibitors afatinib or dacomitinib was reported to also overcome lorlatinib resistance caused by NRG1/ErbB3 activation in ALK-rearranged lung cancer cells in absence o other secondary *ALK* mutations (77). These last findings are very promising, since both pan-ErbB inhibitors have already been approved in the clinical setting for patients with EGFR-mutated NSCLC (NCCN Guidelines for Non-Small Cell Lung Cancer, version 3.2023).

Remarkably, NRG1 fusions have been sporadically reported to be coexistent with ALK fusions in NSCLC patients both in primary and in metastatic sites, whereas pErbB3 and NRG1 high expression significantly correlated with brain metastases from primary lung tumors (2,19,20,78).

Actionable *NRG1* fusions were also listed as acquired oncogenic fusions among those that emerged from a wide screening of a large cohort of EGFR mutant patients under EGFR-TKI treatment having exon19 microdeletions or p.Leu858Arg mutations in exon 21 (53).

Conclusions

Ever increasingly and consistent data suggest that the fusions of *NRG1* gene could represent a novel agnostic and promising marker for therapy in lung IMA and all NRG1-positive tumors and oncogene-addicted NSCLC under TKI treatment. Ongoing researches around the world are aimed now at accelerating the translation of all just available information about NRG1 fusions into clinical practice, by increasing the technical and biological knowledges on this topic.

Several main expected outcomes are now demanded. We need to more specifically clarify the epidemiology of NRG1 fusions in uninvestigated and rare variants of tumor histologies and sub-histologies, using a robust methodological diagnostic workflow. In this context, closing the gap for some methodological approaches, as well as the characterization of genomic breakpoints of just identified NRG1 fusions will be of great importance to uncover whether all of only some genomic breakpoints can unequivocally generate in-frame functional fusion transcripts/proteins to accurately select patients for targeted therapy. Moreover, this could provide the basis to also design a more effective and rigorous DNA-based approach for the detection of NRG1 fusions in biological samples (tissues and liquid biopsies).

Finally, since the activation of the NRG1/ErbB signal may impact on therapy response of lung IMAs and lung adenocarcinomas progression during targeted therapy with TKIs, more larger studies obtained by evaluating the impact of *NRG*s rearrangements on response TKI-treated patients will clarify the predictive value of NRG1 fusions. The optimization of cfRNA analysis to detect the various NRG1 fusions could expand the number of patients to screen, taking into account that many IMA patients are frequently inoperable and that TKI resistance is often detected by liquid biopsy. In this context, a more better definition of the biological context related to NRG1 fusion variants and NRG/ErbB pathway deregulation by functional analyses will expand the selection of molecules that could inhibit the oncoligand-receptor binding activity. This aspect ultimately will lead to novel therapeutic targets and pharmacological approaches to overcome TKI resistance in lung adenocarcinoma patients.

The achievement of all these objectives will allow to rapidly extend the opportunity of personalized medicine to patients with rare and aggressive lung cancer histologies and implement the panel of targetable molecular markers, useful to select NSCLC carrier patients and to design future clinical trials.

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Footnote

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Supplementary

Table S1 The search strategy summary

Items	Specification
Date of search	9 January 2023
Databases and other sources searched	PubMed, clinicaltrials.gov
Search terms used	"NRG1", "ErbB3 AND NRG1", "lung cancer AND NRG1", "NRG1 fusion", "NRG1 rearrangement" "invasive mucinous adenocarcinoma AND NRG1 fusion", "NRG1 AND cancer", "NRG1 fusion detection", "TKI AND NRG1"
Timeframe	Scientific articles from From January 1992 to 2023 were selected. Release from clinicaltrial.gov are to 2023
Inclusion and exclusion criteria	Only peer-reviewed texts published in English language were selected. No additional restrictions
Selection process	Selection was made by the author

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