NECTIN4 amplification (amp) as an actionable alteration in non-small cell lung cancer (NSCLC)

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Johannes Brägelmann[1,2], Niklas Klümper[3,4], Alexander Azizi[5], Emma Colliver[5], Christiane Kümpers[6], Christian Watermann[6], Carsten Heidel[6], Sven Perner[7], Finn-Ole Paulsen[6], Margarete Odenthal[8], Reinhard Buettner[8], Jürgen Wolf[9], Christoph Kuppe[10], Arndt Hartmann[11], Manuel Ritter[3], Viktor Grünwald[12], Michael Hölzel[4], Charles Swanton[5], Markus Eckstein[11]

[1]Translational Genomics, University of Cologne, Cologne, Germany; [2]Medical Clinic III, University Hospital Bonn, Bonn, Germany; [3]Department of Urology, University Hospital Bonn, Bonn, Germany; [4]Institute of Experimental Oncology, University Hospital Bonn, Bonn, Germany; [5]Cancer Evolution and Genome Instability Laboratory, The Francis Crick Institute, Cancer Genome Evolution Research Group, Cancer Research UK Lung Cancer Centre of Excellence, University College London Cancer Institute, London, UK; [6]Institute of Pathology, University Hospital Schleswig-Holstein, Campus Lübeck, Lübeck, Germany; [7]Pathora Institute, Reutlingen, Germany; [8]Institute of Pathology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany; [9]Department of Internal Medicine I, University Hospital Cologne, Cologne, Germany; [10]Division of Nephrology and Clinical Immunology, RWTH Aachen University Hospital, Aachen, Germany; [11]University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; [12]Institute for Medical GU Oncology, Essen University Hospital, Essen, Germany

Introduction

- *NECTIN4* is expressed in a range of solid tumours, including urothelial carcinoma and breast cancer [1,2]
- *NECTIN4* amp has emerged as a promising predictive biomarker for Nectin-4–targeting therapies [3]
- In NSCLC, the Nectin-4–targeting Bicycle® Drug Conjugate, zelenectide pevedotin, showed a 40% response rate in *NECTIN4* amplified vs. 0% in *NECTIN4* non-amplified patients in the Duravelo-1 trial (NCT04561362) [4]
- Zelenectide pevedotin (prev. BT8009 [5]) is currently being investigated in Duravelo-4 (NCT06933329) for previously treated patients with *NECTIN4* amplified NSCLC following its FDA Fast Track Designation
- Currently, systematic analyses of Nectin-4 expression, *NECTIN4* amp, and its genomic context in NSCLC are lacking

⇒ Here, we elucidate the biological and clinical significance of *NECTIN4* amp using tissue microarrays (TMAs) and genomic datasets across multiple patient cohorts

Methods

- TMAs from three academic centres (University Hospitals Cologne, Lübeck, and Erlangen), with N=583 NSCLC tissue samples, were assessed for *NECTIN4* copy number by FISH: amp (*NECTIN4*/*CEN1* ratio >2.0), polysomy (*NECTIN4*/*CEN1* ratio <2.0, copy number >6.0), low-level gain (copy number <6.0), or diploid
- Membranous Nectin-4 protein expression was measured by immunohistochemistry (IHC)
- Whole-exome sequencing (WES) and RNA-seq were performed on N=106 NSCLC samples
- NSCLC samples were analysed for *NECTIN4* amp status, clinical parameters, co-occurring mutations, transcriptional profiles, and immune infiltration using bulk deconvolution by CIBERSORTx
- Co-expression of *NECTIN4* with clinically relevant targets and immune cell subsets was validated in RNA-seq data of lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) cohorts from The Cancer Genome Atlas (TCGA)

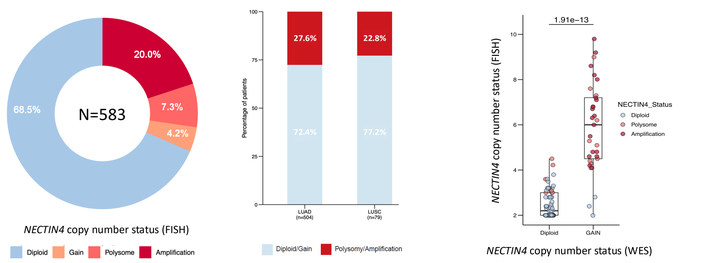
Results

NECTIN4 is frequently amplified in NSCLC

- In a multi-centre cohort of patients with NSCLC (N=583 samples), *NECTIN4* amp and polysomy were detected by FISH in 20.0% and 7.3% of samples, respectively
- The frequency of copy number increase (amp or copy number gain) was comparable in LUAD (n=504) and LUSC (n=79) samples
- In the sub-cohort with WES data (n=106), concordance was high for detection of *NECTIN4* copy number status between WES and FISH (p<0.001)
- Samples with *NECTIN4* copy number increase according to WES had significantly higher *NECTIN4* copy number by FISH

Comparison of *NECTIN4* CN by WES vs. FISH in Erlangen NSCLC cohort

Characteristic	N	Diploid (n=60)	Polysome (n=16)	Amp (n=20)	p-value
WES_N4_CNV	96				
Diploid		57 (95%)	6 (38%)	0 (0%)	p<0.001
GAIN		3 (5%)	10 (63%)	20 (100%)	

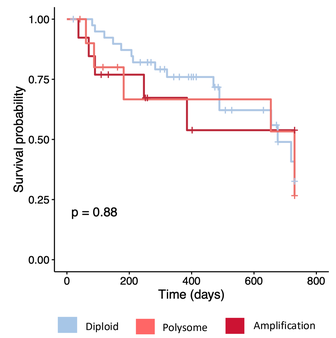


Clinical associations in Erlangen NSCLC cohort (n=106)

- In our Erlangen NSCLC cohort with WES data, we observed significant associations of *NECTIN4* copy number status with *KRAS* mutation and smoking history, but not with other clinically relevant parameters
- Survival did not differ by *NECTIN4* copy number (polysomy/amp vs. diploid/gain)

Characteristic, n (%)	Diploid (n=66)[a]	Polysome (n=18)[a]	Amp (n=22)[a]	p-value[a]
Age, median (Q1, Q3)	65 (58, 72)	63 (56, 68)	67 (61, 71)	0.5
Sex				0.5
Male	42 (64)	9 (50)	15 (23)	
Female	24 (36)	9 (50)	7 (18)	
Smoking Status[b]				0.043
Never	11 (17)	0	1 (5)	
Previous	36 (55)	8 (44)	16 (73)	
Active	17 (26)	10 (56)	5 (23)	
T staging at diagnosis[b]				0.9
T1	8 (12)	2 (11)	2 (9)	
T2	12 (18)	3 (17)	4 (18)	
T3	5 (8)	3 (17)	4 (18)	
T4	39 (59)	10 (56)	12 (55)	
N staging at diagnosis[b]				0.7
N0	13 (20)	5 (28)	4 (18)	
N1	6 (9)	3 (17)	1 (5)	
N2	19 (29)	3 (17)	9 (41)	
N3	26 (39)	6 (33)	8 (36)	
M staging at diagnosis[b]				0.7
M0	8 (12)	3 (17)	4 (18)	
M1	56 (85)	15 (83)	18 (82)	
Mutation status[c]				
EGFR	2 (3)	0	0	>0.9
KRAS	15 (23)	2 (11)	11 (50)	0.041
ALK	1 (2)	0	0	>0.9
ROS1	2 (3)	1 (6)	0	0.4

[a]Kruskal-Wallis rank sum test; Pearson's Chi-squared test; Fisher's exact test. [b]2 diploid patients had unknown smoking status and TNM staging at time of diagnosis; 1 polysome patient had unknown N staging at time of diagnosis.[c]5 diploid patients had unknown ALK and ROS1 mutation status, 6 diploid patients had unknown EGFR and KRAS status; 6 polysome patients had no known mutation status; 1 amp patient had no known mutation status.



NECTIN4 expression levels correlate with differential expression of surface targets and lower immune infiltration

- Across multiple NSCLC cohorts (our own Erlangen NSCLC cohort, TCGA LUAD, and TCGA LUSC), *NECTIN4* expression correlated with high expression of *ERBB2*, *ERBB3,* and *TROP2*
- In contrast, *AXL*, *PDCD1,* and *CTLA4* showed negative correlations with *NECTIN4* expression
- Using bulk deconvolution of RNA-seq with ESTIMATE showed lower immune scores with higher *NECTIN4* expression levels, indicating a distinct (colder) tumour microenvironment in *NECTIN4*-high tumours
- Similarly, CIBERSORTx revealed lower levels of CD8-positive cytotoxic T cells with high *NECTIN4* expression

NECTIN4 copy number correlates with Nectin-4 expression

- Across TMA samples, membranous Nectin-4 protein expression by IHC increased with *NECTIN4* low-level gain (copy number <6), polysomy, and amp
- Similarly, absolute *NECTIN4* copy number determined by FISH correlated significantly with levels of membranous Nectin-4 protein expression (Spearman rho=0.67, p=7x10^{-83})
- In patients with WES and RNA-seq data available, *NECTIN4* copy number increase according to WES was associated with significantly higher *NECTIN4* RNA expression

NECTIN4 copy number increase correlates with lower expression of inflammatory and EMT gene sets

- Differential gene expression analysis (RNA-seq) from our Erlangen cohort highlighted substantial transcriptional differences when separating groups by those with *NECTIN4* copy number increase (polysomy/amp) vs. those without (diploid/gain)
- Gene set enrichment analysis (GSEA) using the MIT Broad MSigDB Hallmark gene sets showed that high *NECTIN4* copy number correlates with increased expression of genes involved in metabolic processes, such as cholesterol homeostasis and MTORC1 signalling
- In contrast, transcriptional signatures related to Epithelial-Mesenchymal Transition (EMT) and inflammation (ALLOGRAFT_REJECTION, IL6_JAK_STAT3, and TNFA_SIGNALLING) were significantly reduced

Conclusions

- *NECTIN4* copy number is increased (polysomy/amp) in ~20–30% of NSCLC samples and correlates with increased protein and RNA expression
- *NECTIN4*-high NSCLC tumours form a distinct subgroup with low EMT and inflammatory gene signalling
- Differences in immune infiltration and expression of clinically relevant surface targets highlight potential relevance for clinical treatment strategies and therapy response

⇒ Considering the enhanced efficacy of zelenectide pevedotin in *NECTIN4*-amp NSCLC in Duravelo-1, these findings suggest *NECTIN4* amp as a promising predictive biomarker for patient stratification and advanced precision oncology in NSCLC

References

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Abbreviations

amp, amplification; *CEN1*, centromere 1; CN, copy number; CNV, copy number variation; EMT, epithelial mesenchymal transition; FISH, fluorescence in situ hybridisation; GSEA, gene set enrichment analysis; IHC, immunohistochemistry; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MIT, Massachusetts Institute of Technology; NSCLC, non-small cell lung cancer; TCGA, The Cancer Genome Atlas; TMA, tissue microarray; TPM, transcripts per million; WES, whole exome sequencing

Presenting author: Johannes Brägelmann, johannes.braegelmann@uni-bonn.de

Potential conflicts of interest of presenting author:
JB has received travel funds from Bicycle Therapeutics and Merck KG and research funding from Bayer AG.

Presented at the European Society for Medical Oncology (ESMO) Congress, Berlin, Germany, 17–21 October 2025