



# BRD4-NUT Fusion Oncogene Analysis by RT-PCR in Sinonasal Undifferentiated Carcinoma (SNUC) and Other Aggressive Primary Sinonasal Malignancies: a Study of 15 Cases.

JJ Garcia<sup>1</sup>, K Nafa<sup>2</sup>, DL Carlson<sup>2</sup>, LA Johnson<sup>3</sup>, EL Barnes<sup>1</sup>, EB Stelow<sup>4</sup>, RR Seethala<sup>1</sup>, CA French<sup>3</sup>, M Ladanyi<sup>2</sup>

<sup>1</sup>Pathology and Laboratory Medicine, University of Pittsburgh, Pittsburgh, PA, United States; <sup>2</sup>Department of Pathology, Memorial Sloan-Kettering Center, New York City, NY, United States; <sup>3</sup>Department of Pathology, Brigham and Women's Hospital, Boston, MA, United States; <sup>4</sup>Department of Pathology, University of Virginia, Charlottesville, VA, United States

## Introduction

*NUT*-rearranged carcinomas (NRCs), also termed *NUT* midline carcinomas, have been characterized as poorly differentiated carcinomas of midline anatomic location that affect young patients. NRCs almost invariably follow a lethal clinical course.

Approximately 2/3 of NRCs reported in the literature are characterized by a balanced chromosomal translocation represented by t(15;19) resulting in a *BRD4-NUT* fusion gene.

The remaining 1/3 of NRC cases, so-called *NUT*-variant carcinomas, involve other fusion partners such as *BRD3*, another bromodomain gene.

*NUT* rearrangements have been identified using fluorescent *in-situ* hybridization (FISH) in a subset of undifferentiated carcinomas of the sinonasal tract—including sinonasal undifferentiated carcinoma (SNUC)—in older patients.

This study sought to explore the incidence of *NUT* rearrangement in cases of SNUC and other primary sinonasal malignancies. Moreover, reverse transcriptase polymerase chain reaction (RT-PCR) was employed to investigate whether *NUT* rearrangement in such cases involve *BRD4* as a fusion partner.

## Methods

### Case Selection and Clinical Findings

Fifteen cases of poorly differentiated sinonasal carcinoma were retrieved from the archives of the University of Pittsburgh Medical Center and Memorial Sloan-Kettering Cancer Center. Clinical parameters were recorded when available.

### Morphologic Parameters

Hematoxylin and eosin (H&E) stained sections were reviewed in all cases. CD34 (Ventana Medical Systems, Tucson, AZ) and anti-*NUT* monoclonal rabbit antibody (French et al, Boston, MA) were available for review in 11 and 6 cases, respectively.

### Reverse transcriptase Polymerase Chain Reaction

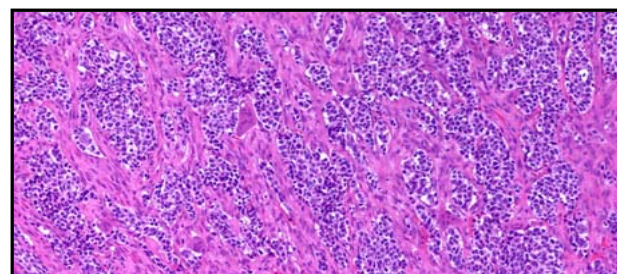
RT-PCR was performed using the Qiagen (Valencia, CA) one-step RT-PCR kit as described. Two primer sets were used, BR2276F/*NUT*1194R and BR2334F/*NUT*1132R, identical to those used previously. PCR products were electrophoresed on a 2.5% agarose gel stained with ethidium bromide in 1x Tris acetic acid ethylenediamine tetraacetic acid (TAE) buffer. Positive controls were from *BRD4-NUT* cell line RNA, TC-797 and 690. To assess the adequacy of the extracted RNA for analysis, RT-PCR was also performed using primers spanning an intron of the ubiquitously expressed phosphoglycerate kinase (*PGK*) gene, resulting in amplification of a 263-bp fragment. RNA samples in which the *PGK* product could not be demonstrated were considered inadequate for *BRD4-NUT* analysis (8 cases eliminated from study).

### Fluorescent *In situ* Hybridization

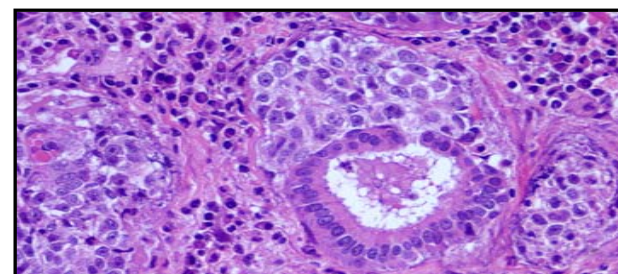
Dual-color FISH assays were performed on formalin-fixed, paraffin-embedded 4- $\mu$ m tissue sections as described. Probes used for the 15q14 *NUT* break-point, flanking a 181-kb region containing *NUT*, included 3' telomeric BAC clones 1H8 and 64o3, and 5' centromeric clones 412e10 and 3d4. Positive controls were metaphase preparations from the *BRD4-NUT* cell line, TC-797. Sections in which >80% of cells contained hybridization signals in 4 areas (>200 cells/area) were considered adequate for interpretation.

## Results

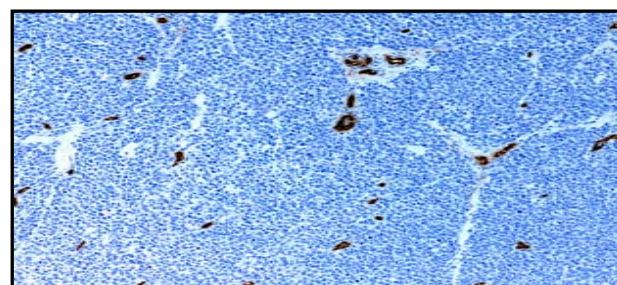
Case	Age	Sex	Diagnosis	BRD4-NUT (RT-PCR)	NUT Rearranged (FISH)	NUT IHC	CD34 IHC
1	68	M	SN Undifferentiated Carcinoma	Negative	Negative	Negative	Negative
2	79	F	PD Carcinoma	Negative			Negative
3	57	F	SN Undifferentiated Carcinoma	Negative	Negative	Negative	Negative
4	50	M	SN Undifferentiated Carcinoma	Negative	Negative	Negative	Negative
5	64	F	PD Squamous Cell Carcinoma	Negative			Negative
6	69	M	SN Undifferentiated Carcinoma	Negative			
7	36	M	PD Carcinoma	Negative			Negative
8	79	F	SN Undifferentiated Carcinoma	Negative	Negative	Negative	Negative
9	59	M	MD Squamous Cell Carcinoma	Negative			Negative
10	48	M	SN Undifferentiated Carcinoma	Negative	Negative	Negative	Negative
11	69	M	SN Undifferentiated Carcinoma	Negative	Negative	Negative	
12	65	F	PD Squamous Cell Carcinoma	Negative			Negative
13	69	M	Lymphoepithelial Carcinoma	Negative			
14	49	F	PD Carcinoma	Negative			Negative
15	58	F	SN Undifferentiated Carcinoma	Negative			



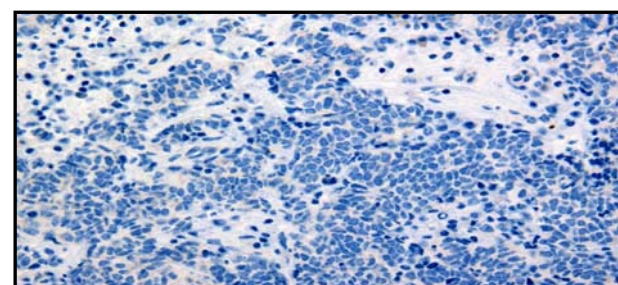
SNUC, Case 6



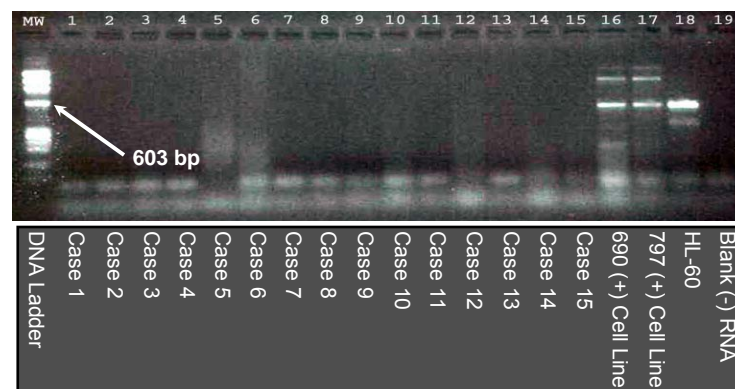
SNUC, Case 1



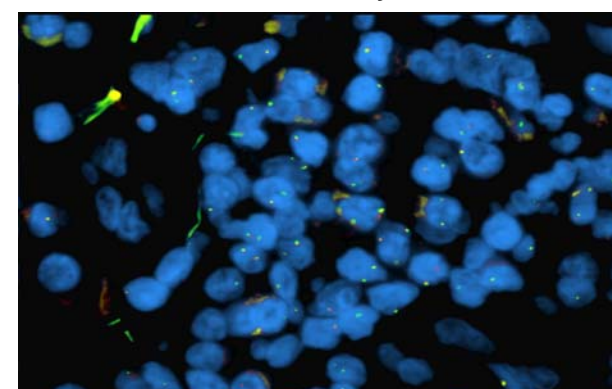
CD34, Case 2



NUT Monoclonal Antibody, Case 1



BRD4-NUT fusion transcript cDNA (RT-PCR)



NUT split-apart FISH, Case 1

- Eight of 15 cases diagnosed as SNUC; remaining cases classified as poorly differentiated carcinoma (3), squamous cell carcinoma (3) and lymphoepithelial carcinoma (1).

- Patients were predominantly adults (median 61.3 years; range 36-79 years).

- RT-PCR for *BRD4-NUT* fusion transcript was uniformly negative in all cases (0/15, 0%).

- FISH for *NUT* rearrangement was negative in all tested cases (0/6, 0%).

- NUT immunohistochemical staining was negative in all tested cases (0/6, 0%).

- CD34 immunohistochemical staining was negative in all tested cases (0/11, 0%).

## Conclusions

- Incidence of *NUT* rearrangement is low in poorly differentiated carcinomas of the sinonasal tract, including SNUC.

- Poorly differentiated carcinomas of the sinonasal tract that are negative for the *BRD4-NUT* fusion transcript by RT-PCR should be tested by FISH to exclude the possibility of a *NUT* variant.

- CD34 negative staining, as previously suggested in the literature, may be a surrogate marker for the *NUT* wild type in poorly differentiated carcinomas of the sinonasal tract.

- Further study of poorly differentiated carcinomas of the sinonasal should be undertaken to determine the incidence and histopathologic features of *NUT*-rearranged carcinomas.

## References

- EB Stelow, AM Bellizzi, K Taneja, SE Mills, RD LeGallo, JL Kutok, JC Aster, CA French. *NUT* Rearrangement in Undifferentiated Carcinomas of the Upper Aerodigestive Tract. *Am J Surg Pathol* 2008; 32:828-834.
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- CA French, JL Kutok, WC Faquin, JA Toretsky, CR Antonescu, CA Griffin, V Nose, SO Vargas, M Moschovi, F Tzortzatou-Stathopoulou, I Miyoshi, AR Perez-Atayde, JC Aster, JA Fletcher. Midline Carcinoma of Children and Young Adults With *NUT* Rearrangement. *J Clin Oncol* 22:4135-4139.