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Taiwan Association of Gynecologic Oncologists

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Tumor Necrosis Factor-alpha Promoter Polymorphisms in Neuroendocrine Adenocarcinoma of the Uterine Cervix

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Purpose of this study

- Presentation of a large series of neuroendocrine cervical cancer with complete F/U data
- Try any of further in-depth molecular investigation in regard to this disease's etiology or clinical course

Neuroendocrine carcinoma of uterine cervix

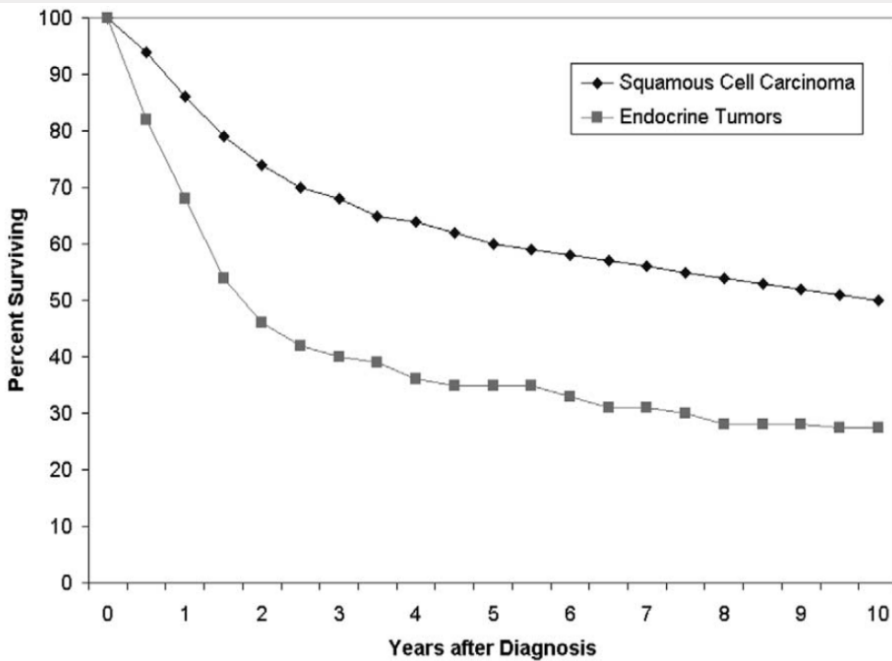
- < 5% of cervical cancer
- Biologically- frequent early nodal & distant metastasis
- Poor prognosis despite of aggressive treatment in most patients
- Endocrine tumor of the uterine cervix (1996 College of American Pathologists and NCI)
 - Typical carcinoid
 - Atypical carcinoid
 - Large cell neuroendocrine
 - Small (oat) cell

Definition of cervical small cell neuroendocrine tumor

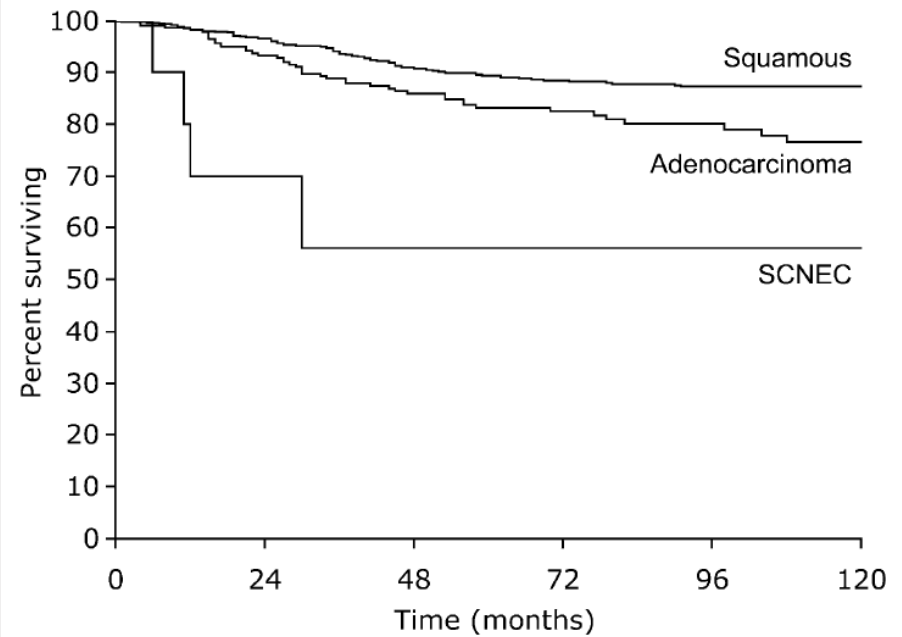
- Histologically indistinguishable from small cell Ca of other sites (WHO definition)
 - characteristics
 - 80% positive for neuroendocrine markers (synaptophysin, chromogranin, NSE, CD56...)
- Small (oat) cell
 - “ a cancer with small round or fusiform cells with scant cytoplasm and hyperchromatic nuclei, finely granular chromatin and absent or inconspicuous nucleoli, numerous mitotic figures and extensive necrosis”
 - IHC stains are not necessary to make the diagnosis

Prognosis of cervical neuroendocrine Ca

All stage (SEER)



Stage Ib



Margaret et al, Gynecologic Oncology, 2003

Viswanathan et al, Gynecologic Oncology, 2004

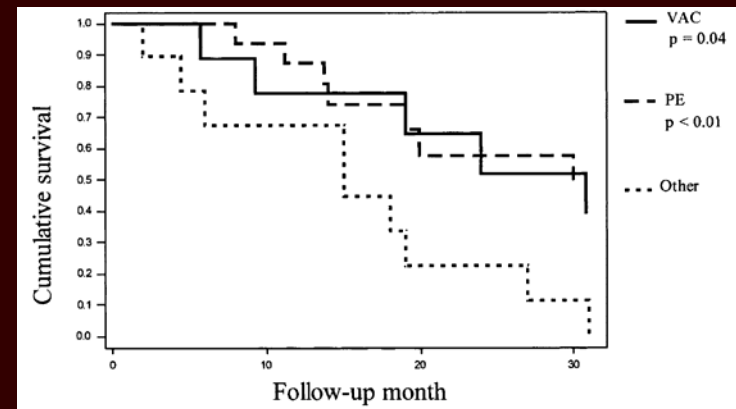
SEER Data

Comparison of patients with endocrine tumors of the uterine cervix versus squamous cell carcinoma of the uterine cervix, SEER data, 1973–1998

Characteristic	Endocrine tumors (<i>n</i> = 239)	Squamous cell carcinoma (<i>n</i> = 18,458)	<i>P</i> value
Age at diagnosis, years			
Mean (SE)	49 (1.1)	52 (0.1)	<0.01
Median	45	50	
FIGO stage (%; 1983–1998) ^a			<0.01
I	81 (42)	5651 (51)	
II	36 (19)	2402 (22)	
III	20 (10)	1395 (12)	
IV	44 (23)	853 (8)	
Unknown	11 (6)	814 (7)	
Type of surgery (%; 1983–1998) ^a			0.4
Pelvic exenteration	0 (0)	51 (<1)	
Radical hysterectomy	56 (29)	2775 (25)	
Simple hysterectomy	24 (13)	1610 (14)	
Local tumor excision/destruction	98 (51)	5545 (50)	
No surgery	9 (5)	654 (6)	
Other	5 (2)	480 (4)	
Lymph nodes involved (%; 1983–1998) ^a			<0.01
Yes	71 (57)	1363 (18)	
No	54 (43)	6179 (82)	
Lymph node involvement unspecified/unknown	67	3573	
Therapy (%)			
Radiation	143 (60)	11629 (63)	0.3
Chemotherapy	103 (43)	1608 (9)	<0.01

Treatment for cervical small cell neuroendocrine carcinoma

- Standard treatment recommendations have **yet to be established**
rarity, diagnosis definition, no prospective trial
- Most favor Tx with C/T? Multi-modality Tx preferred
 - RH (primary or adjuvant setting?)
 - C/T based on Tx for SCLC - VAC, P(A)E or Taxol + Carboplatin.....
 - R/T for loco-regional
 - Concurrent RT and C/T
 - Prophylactic cranial R/T optional?



	Study year	No. of pts	% stage 1	Local treatment (%)			Chemotherapy (%)	FFS (%)	Median OS (M)	Dead (No)
				RT	Surgery	Both				
Van Nagell	1988	25	48	52	48	0	0	36	60	16
Sheets	1988	14	86	0	43	57	0	14	--	12
Walker	1988	14	29	78	14	50	21	8	12	--
Gersell	1988	15	60	73	6	26	33	33	11	13
Miller	1991	14	43	42	21	28	50	--	9	8
Morris	1992	10	70	70	20	10	100	40	28	5
Chang	1993	28	--	some	most	some	some	--	10	17
Abeler	1994	26	58	19	46	34	some	11	--	22
Sevin	1996	12	83	0	42	58	0	36	20	--
Sykes	1999	11	--	63	18	18	45	36	--	7
Boruta II	2001	19	86	77	52	37	58	42	21	8
Hoskins	2003	31	52	81	0	13	100	54	36	10
Viswanathan	2004	21	75	71	24	0	66	28	30	15
Lee	2008	68	85	70	47	29	41	--	54	--
Zivabivuc	2009	17	88	37	100	54	41	29	14	12

Prognosis of Small cell neuroendocrine carcinoma of uterine cervix

- Prognosis factor?

 - Stage

 - Tumor size


 - LN status

 - Histological pattern

 - HPV and type?

 - Adjuvant treatment

- Molecular marker?

- 
- Multi-institutional study (MMH, CGMG, VGH)
 - 17-year period (1991 – 2007)
 - 102 neuroendocrine cervical cancer (91 SCNEC, 4 ACT, 7 LCNEC)
 - IRB approval
 - HPV determination
 - DNA for other molecular study

Clinical profiles and related statistics of NECC patients ($n = 102$)

	SCNEC	LCNEC	ACT	Total
<i>n</i>	91	7	4	102
Age (yr)	49.2±14.5	42.3±10.9	44.3±4.6	48.5±14.1
FIGO stage				
IA1	1	0	0	1
IA2	0	1	0	1
IB1	34	4	3	41
IB2	19	2	0	21
IIA	8	0	0	8
IIB	11	0	0	11
IIIB	6	0	1	7
IVA	3	0	0	3
IVB	9	0	0	9
Histologic Pattern:				
Pure	69	4	1	74
Mixed	22	3	3	28
HPV Status:				
HPV-16	7	0	1	8
HPV-18	47	6	2	55
HPV-30	1	0	0	1
HPV-33	1	0	0	1
HPV-52	1	0	0	1
HPV(-)	15	1	1	17
HPV(NR)	19	0	0	19

Clinical profiles and related statistics of NECC patients ($n = 102$)

	SCNEC	LCNEC	ACT	Total
<i>n</i>	91	7	4	102
LN _p Status:				
LN _p meta (+)	19	3	1	23 (40%)
LN _p mean no.	24.1±12.	20.0±12.1	24.0±1.83	23.7±12.3
LN _p meta (-)	28	4	3	35
LN _p (NR)	42	0	0	42
LN _{pA} Status:				
LN _{pA} meta (+)	3	2	0	5 (9.3%)
LN _{pA} mean no.	2.53±5.67	1.00±1.73	1.00±1.41	2.28±5.23
LN _{pA} meta (-)	42	3	4	49
LN _{pA} (NR)	46	2	0	48
Tumor size (cm)	4.21±2.01	3.07±1.17	3.18±0.89	4.08±1.95 ^a
Adjuvant treatment after RH and BPLD:				
No Tx	9	2	1	12
CT	23	2	1	26
RT	4	0	0	4
CT+RT	15	3	1	19
Overall survival (mos):				
<i>n</i>	90 ^b	7	4	101 ^b
Median (min-max)	21.1(1-232.2)	17.2(3-114)	13.5(4-26)	19.6(1-232.2) ^b
Dead (mos):				
<i>n</i>	51 ^b	5	4	60 ^b
Median (min-max)	14.4(1-120)	11.8(3-39)	13.9(4-26)	14.0(1-120) ^b

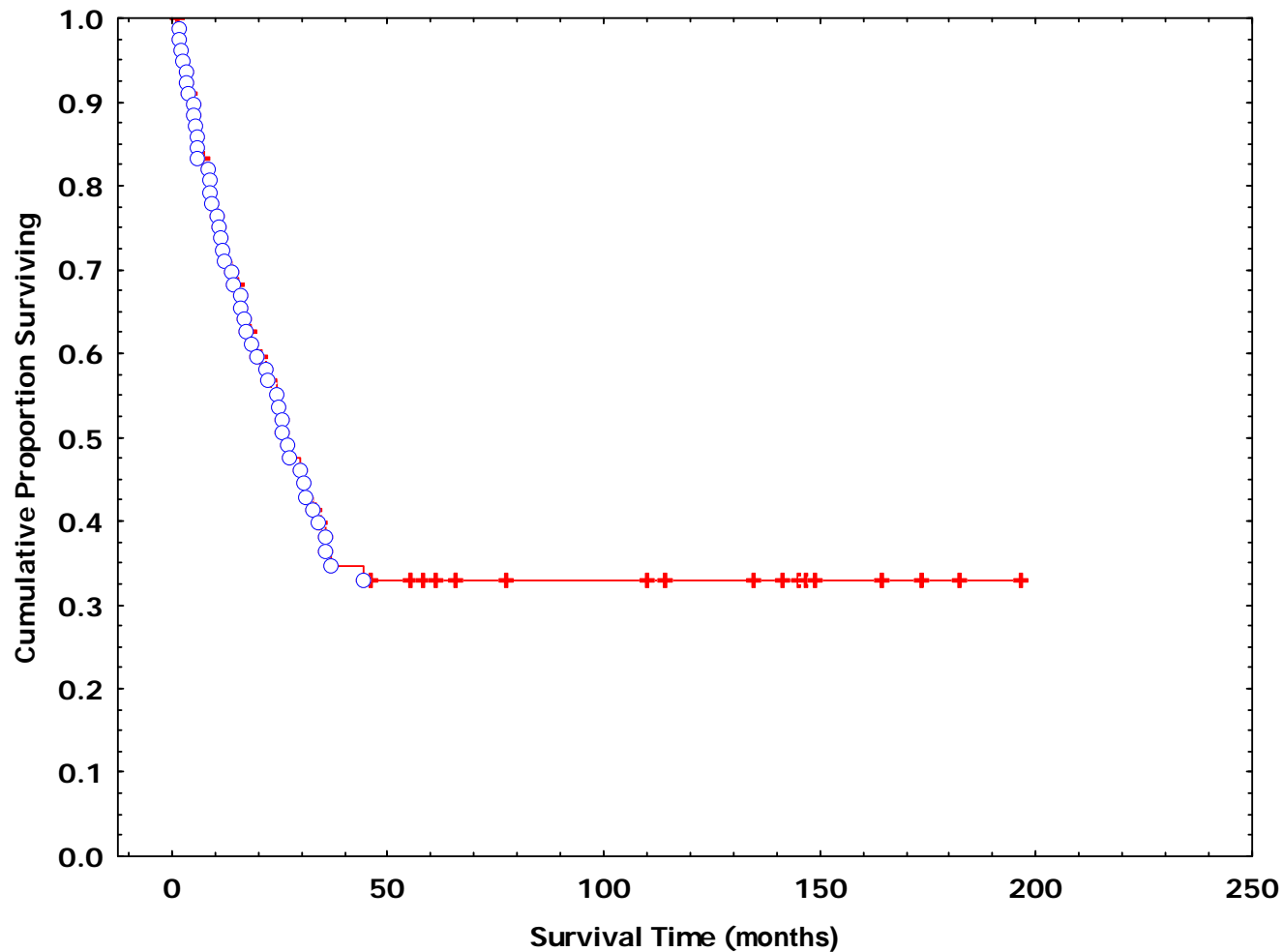
Patients with confirmed LN_p had mostly stage IB diseases [IB1 (43.5%), IB2 (34.8%)]

HPV types distribution in NECC patients and control squamous cell cervical cancer patients.

HPV type	Histology	
	No. Squamous Ca (%)	No. Neuroendocrine Ca (%)
HPV16	50 (51.55)	10 (10.20)
HPV18	7 (7.22)	57 (58.76)
HPV30	0 (0)	1 (1.02)
HPV31	4 (4.12)	1 (1.02%)
HPV33	0 (0)	1 (1.02%)
HPV35	1 (1.03)	0 (0)
HPV45	3 (3.09)	0 (0)
HPV52	6 (6.19)	0 (0)
HPV58	11 (11.34)	0 (0)
HPV69	1 (1.03)	0 (0)
ND	14 (14.43)	28 (28.57%)

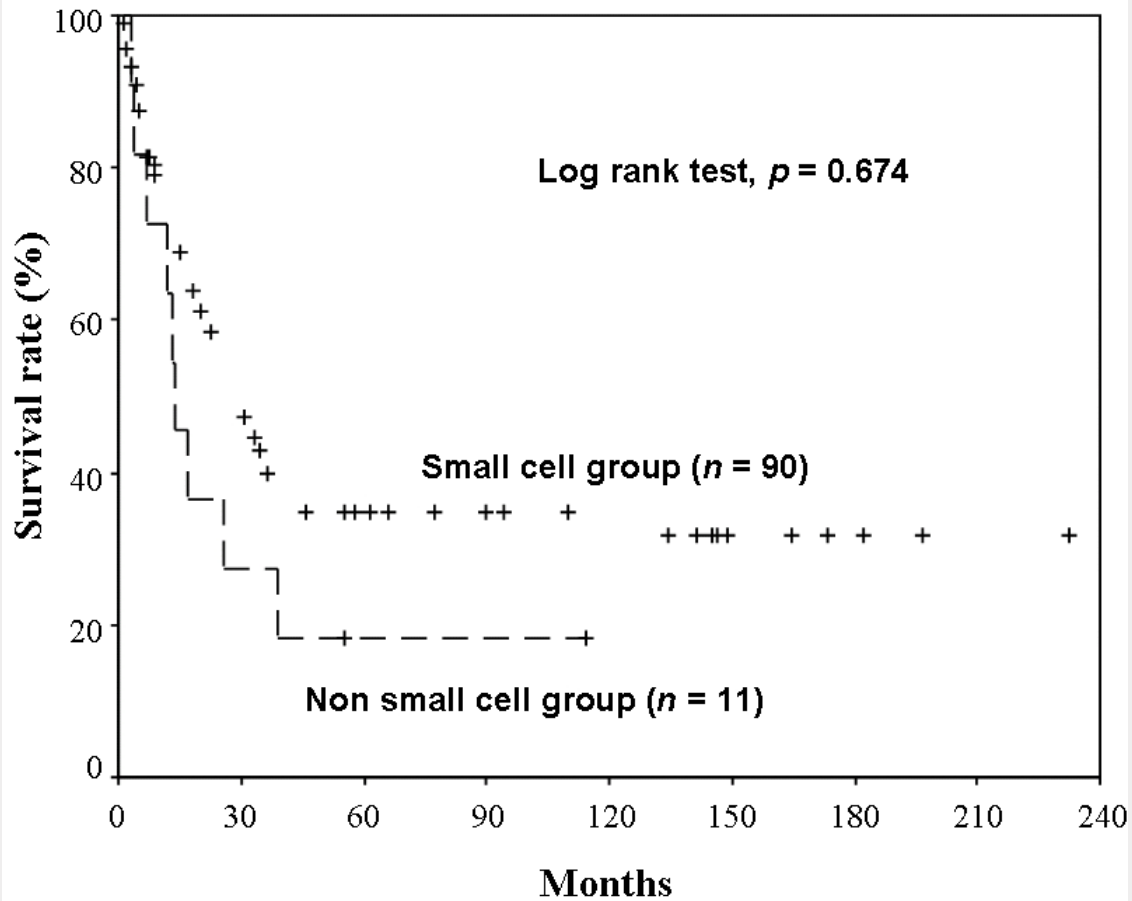
No. of Squamous Ca = 97, Neuroendocrine Ca = 98

Survival analysis shows about 1/3 of NECC patients had relatively long-term survival.



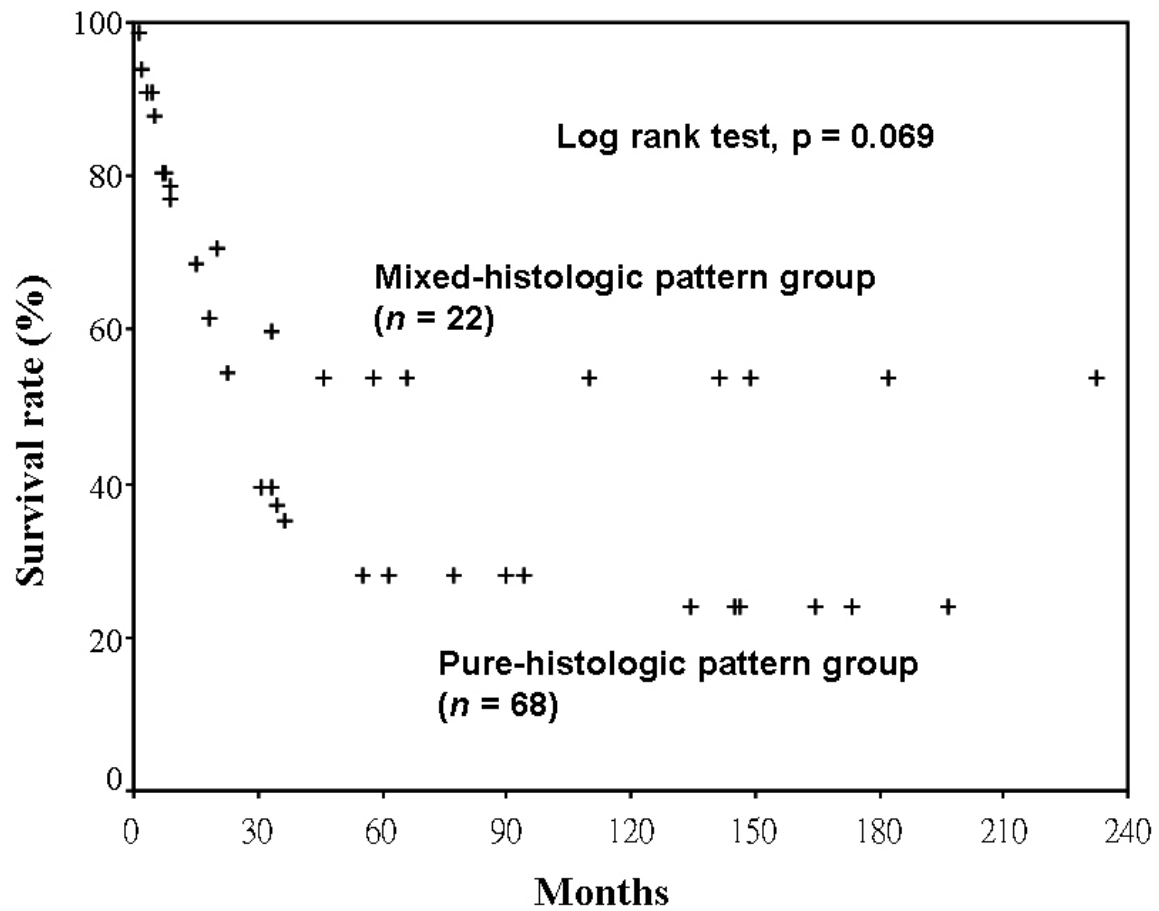
Prognostic factors and survivorship in NECC patients ($n = 102$)

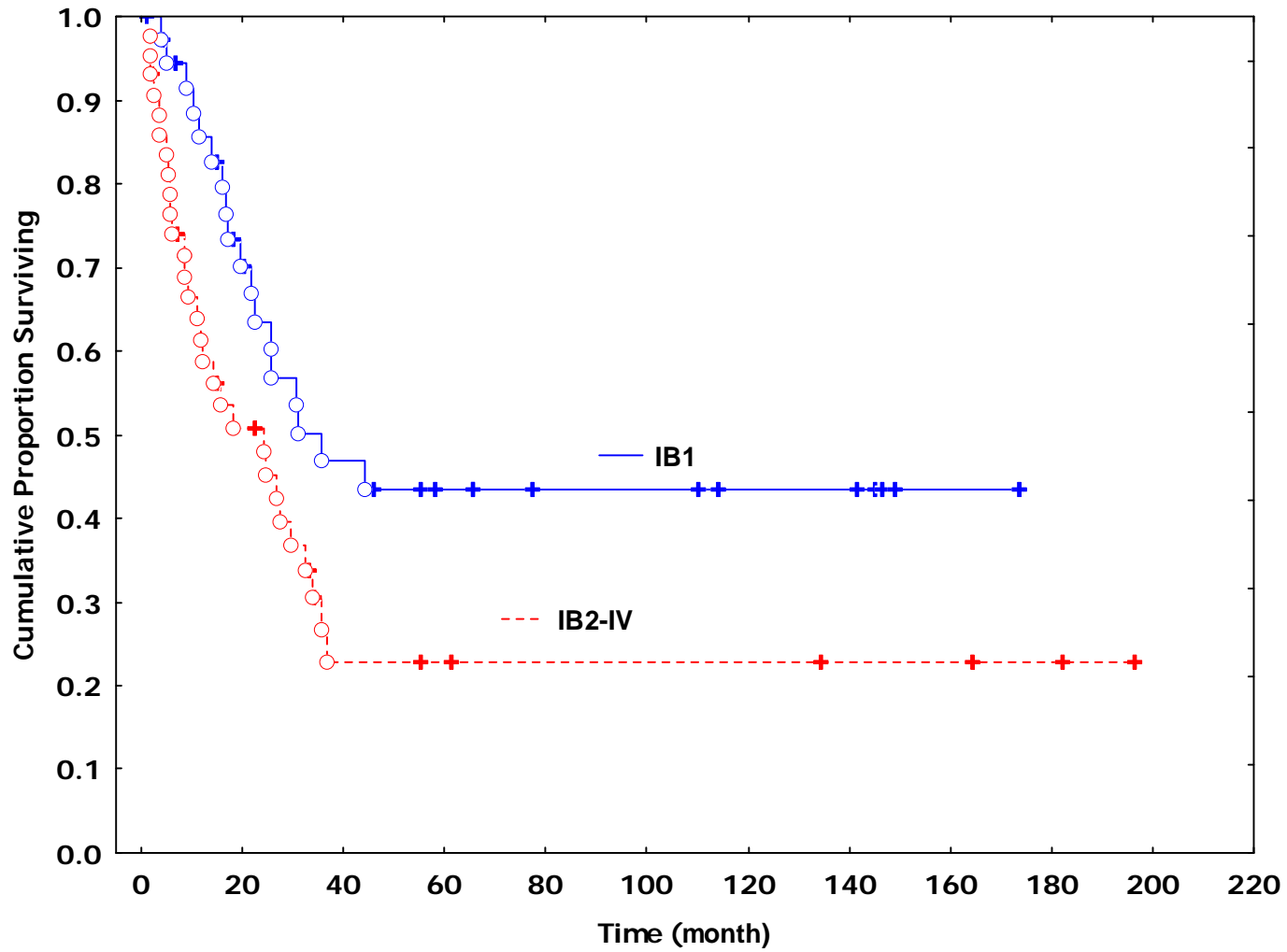
Characteristics	Survivorship (No. survived / No. total)		<i>p</i> value
Age (y/o) ^b			
<30 vs \geq 30	1/5	40/96	0.646
<40 vs \geq 40	13/27	28/74	0.369
<50 vs \geq 50	21/60	20/41	0.216
FIGO stage ^b			
I vs II ($n = 82$)	29/63	11/19	0.437
IB1 vs IB2 ($n = 61$)	21/41	7/20	0.281
\leq IB1 vs $>$ IB1 ($n = 101$)	22/43	19/58	0.069*
Tumor size (cm) ^c			
\leq 1 vs $>$ 1	1/2	38/90	1.000
\leq 2 vs $>$ 2	6/10	33/82	0.314
\leq 3 vs $>$ 3	17/33	22/59	0.196
\leq 4 vs $>$ 4	28/57	11/35	0.129
HPV Status			
HPV-16 vs HPV-18 ($n = 63$)	5/8	21/55	0.257
HPV-18 vs HPV(-) ($n = 72$)	21/55	5/17	0.576
Histologic Pattern ^b			
pure vs mixed	26/73	15/28	0.100
Treatment			
No treatment		2/9	0.092
RH Surgery only ^c		5/12	
RH Surgery with adjuvant CT		17/24	
RH Surgery with adjuvant RT		2/4	
RH Surgery with adjuvant CT+RT		5/16	
Only CCRT(cis-Pt)		8/18	
Only RT		1/6	
LN _p metastasis ^d			
Negative vs Positive	19/35	6/23	0.057*
LN _{pA} metastasis ^e			
Negative vs Positive	24/49	0/5	0.059*

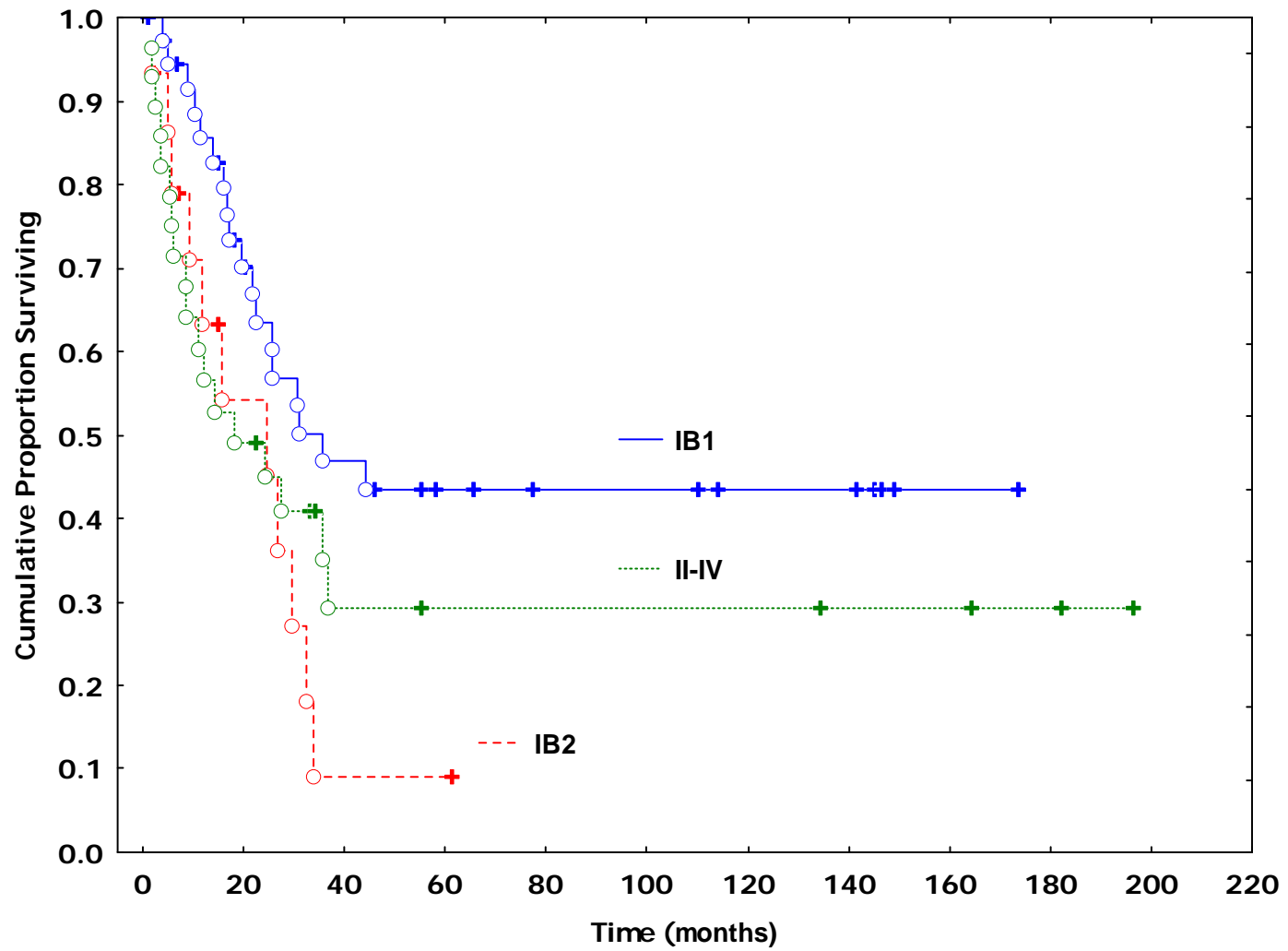


Characteristics, treatment and survivorship of SCNEC patients (n = 91)

Characteristics	Survivorship		p value ^a
	(No. survived / No. total)		
Age (y/o) ^b			
<30 vs ≥30	1/4	38/86	0.630
<40 vs ≥40	12/24	27/66	0.478
<50 vs ≥50	19/51	20/39	0.204
FIGO stage ^b			
I vs II (n = 72)	27/53	11/19	0.789
IB1 vs IB2 (n = 52)	20/34	7/18	0.245
≤IB1 vs > IB1 (n = 90)	20/35	19/55	0.049**
Tumor size (cm) ^c			
≤1 vs >1	1/1	36/80	0.457
≤2 vs >2	6/8	31/73	0.133
≤3 vs >3	15/26	22/55	0.158
<4 vs >4	26/47	11/34	0.046**
HPV Status			
HPV-16 vs HPV-18 (n = 54)	5/7	19/47	0.221
HPV-18 vs HPV(-) (n = 72)	19/47	5/15	0.764
Histologic Pattern ^b			
pure vs mixed	26/68	13/22	0.069
Treatment			
No treatment	2/9		0.113
RH Surgery only ^c	4/9		
RH Surgery with adjuvant CT	16/23		
RH Surgery with adjuvant RT	2/4		
RH Surgery with adjuvant CT+RT	5/15		
Only CCRT(cis-Pt)	8/18		
Only RT	1/6		
LN _p metastasis ^d			
Negative vs Positive	17/28	6/19	0.075*
LN _{pA} metastasis ^e			
Negative vs Positive	22/42	0/3	0.233







Illumina Golden Gate SNP Arrays -Sentrix® BeadChip



Sentrix BeadChip

DNA extraction, QC

SNP array (10 SCC, 10 SCNEC)

6 gene candidate in promoter SNP patterns (IL6, TNF- α ...)

Validation by PCR & RFLP

TNF α -308

TNF-alpha- 308 NcoI digest (107bp)

1 aggcaatagg tttgagggc catggggacg gggttcagcc tccagggtcc tacacacaaa
61 tcagtcagtg gcccaagaaga cccccctcgg aatcggagca gggagga

G to A

primer

TNF-alpha- 308 - F 5`-AGGCAATAGGTTTTGAGGGCCAT-3`

TNF-alpha- 308 - R 5`-TCCTCCCTGCTCCGATTCCG-3`

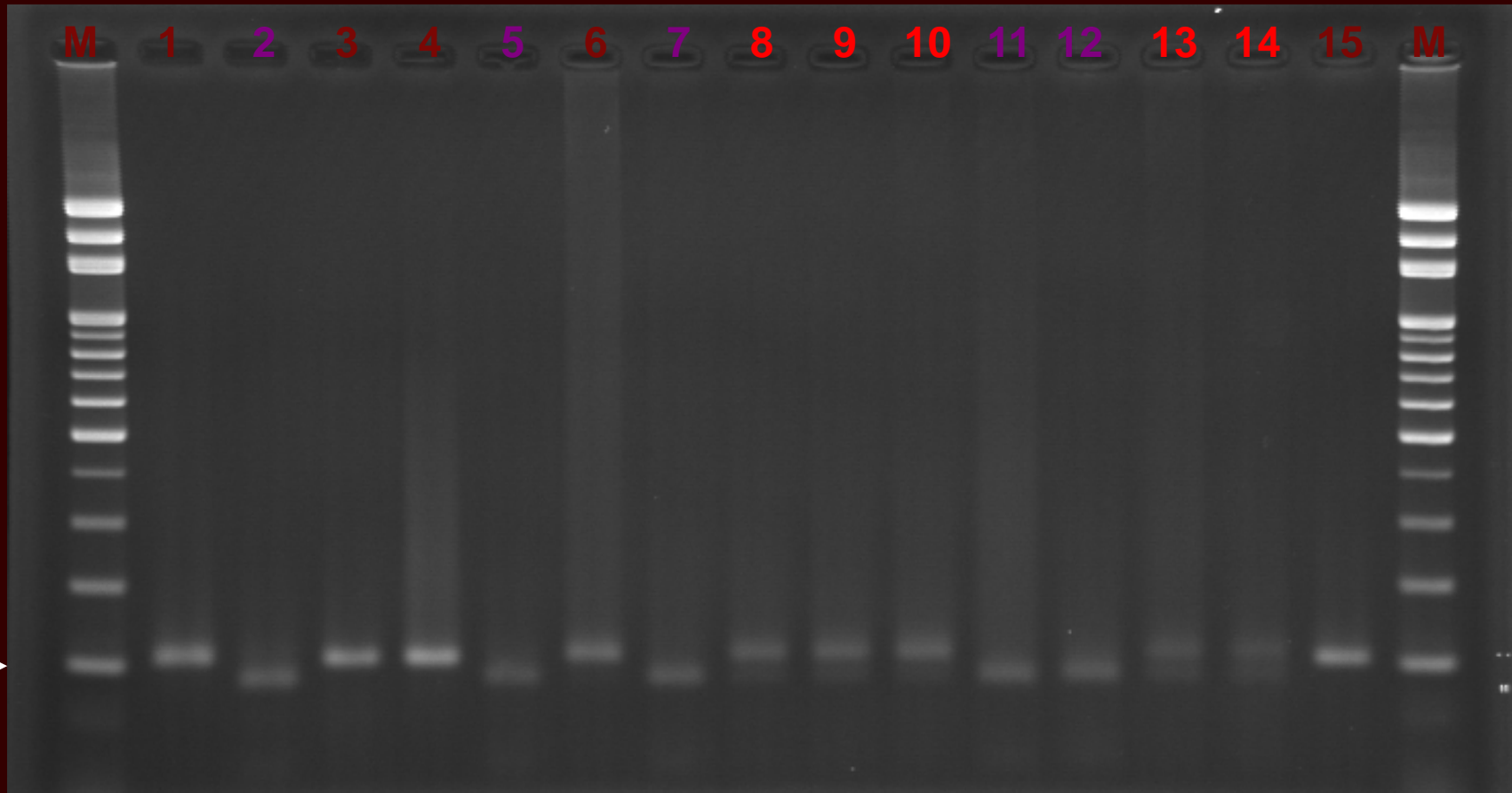
TNF α -308 (Nco I digest)

100 bp
marker

A/A : 107 bp (Lane 1 、 3 、 4 、 6 、 15)

G/A : 107 bp 、 84 bp 、 23 bp (Lane 8 、 9 、 10 、 13 、 14)

G/G : 84 bp 、 23 bp (Lane 2 、 5 、 7 、 11 、 12)



100 bp



TNF α -1031

TNF-alpha-1031 BbsI digest (252bp)

1 acaaggctga ccaagagaga aagaagtagg catgagggat cacagggccc cagaaggcag
61 ggaaaggctc tgaagccag ctgccgacca gagccccaca cggaggcatc tgcacctcg
121 atgaagcca ataaacctct tttctctgaa atgctgtctg cttgtgtgtg tgtgtctggg
181 agtgagaact tcccagtcta tctaaggaat ggagggaggg acagagggct caaagggagc
241 aagagctgtg gggagaaca aaggataagg gctcagagag ctcagggat atgtgatgga
301 ctcaccaggt gaggccgcca gactgctgca ggggaagcaa aggagaagct gagaagatga
361 aggaaaagtc agggctctgga ggggcggggg tcaggagct cctgggagat atggccacat
421 gtagcggctc tgaggaatgg gttacaggag acctctgggg agatgtgacc acagcaatgg
481 gtaggagaat gtccagggct atggaagtcg agtatgggga c

T to C

primer

TNF-alpha- 1031- F 5'-GGCTCAGAGAGCTTCAGGGAT-3'

TNF-alpha- 1031- R 5'-GTCCCCATACTCGACTTCCATA-3'

TNF α -1031 (Bbs I digest)

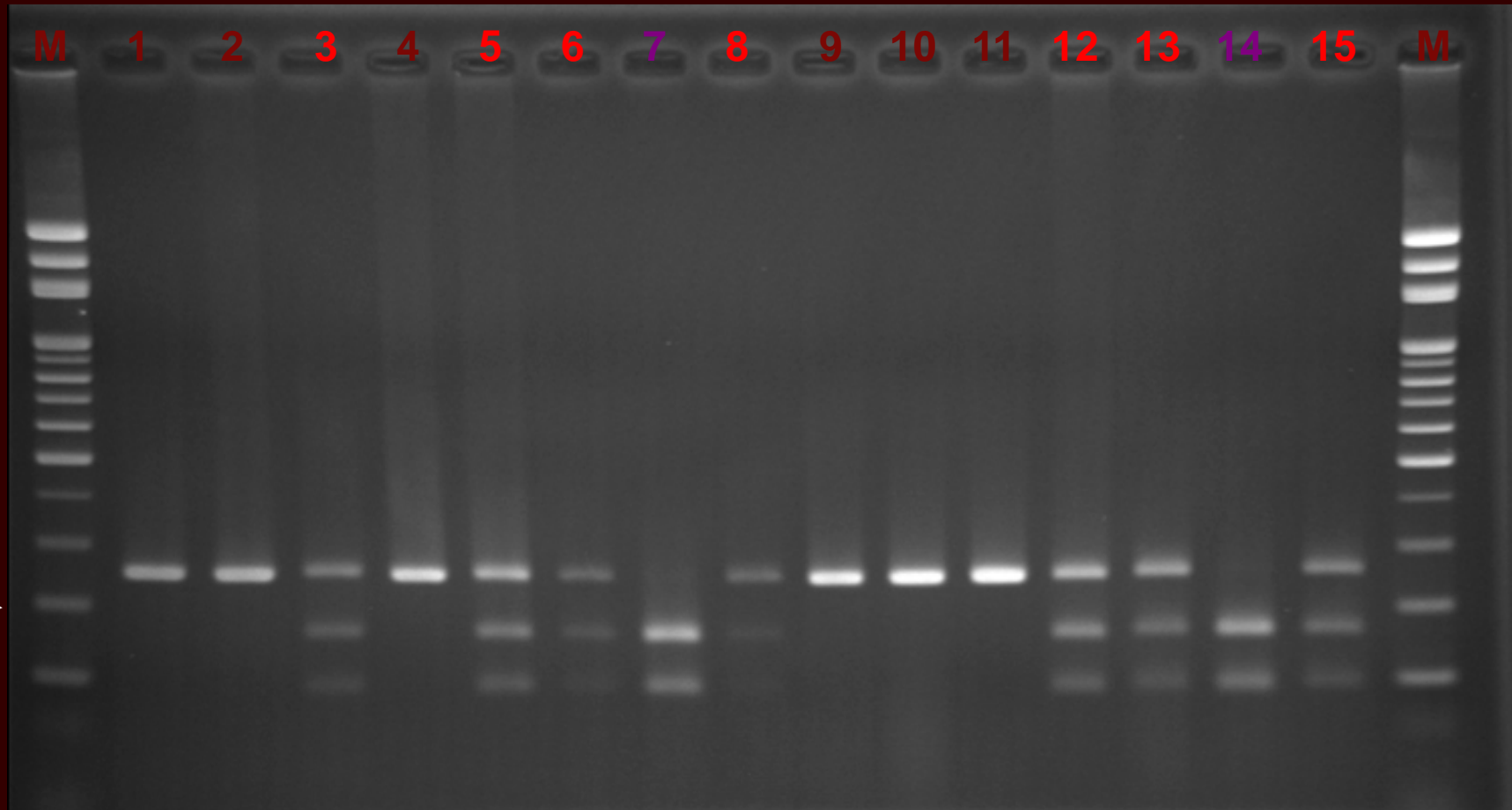
100 bp
marker



C/C : 252 bp (Lane 1 、 2 、 4 、 9 、 10 、 11)

T/C : 252 bp 、 163 bp 、 89 bp (Lane 3 、 5 、 6 、 8 、 12 、 13 、 15)

T/T : 163 bp 、 89 bp (Lane 7 、 14)



TNF α promoter polymorphism (-308 & -1031) in NECC patients, comparing to control squamous cell cervical cancer patients.

TNF α -308(G \rightarrow A)		TNF α -308(G \rightarrow A)		χ^2 , df	<i>p</i> ^a	OR(95% C. I.) ^b
Squamous Ca(97)		Neuroendocrine Ca(98)				
G/G	82(84.54%)	54(55.10%)		$\chi^2=20.554$, df=2	<i>p</i> <0.001	1
G/A	12(12.37%)	39(39.80%)				4.94(2.37-10.27)*
A/A	3(3.09%)	5(5.10%)				2.53(0.58-11.03)
TNF α -1031(T \rightarrow C)		TNF α -1031(T \rightarrow C)		χ^2 , df	<i>P</i> ^a	
Squamous Ca(97)		Neuroendocrine Ca(98)				
T/T	71(72.45%)	47(48.45%)		$\chi^2=16.066$, df=2	<i>p</i> <0.001	1
T/C	23(23.47%)	49(50.52%)				8.52(0.90-80.58)
C/C	4(4.08%)	1(1.03%)				2.65(0.29-24.43)

C. I. : Confidence Interval ; df : degree of freedom

* : *p*<0.05

^a *p* values are based on Chi-Square or Fisher's exact test for categorical variables.

^b Odds Ratio of Mantel-Haenszel Chi-Square test for categorical variables.

Study (reference)	Healthy controls	-1031C allele		-308A allele	
		Freq (%)	Carrier <i>n</i> (%)	Freq (%)	Carrier <i>n</i> (%)
Yen et al ³³	97	NR	NA	14	25 (26)
Wu et al ³⁴	210	15	67 (32)	12	49 (23)
Duan et al ³⁵	340	19	119 (35)	7	45 (13)
Ma et al ³⁶	110	31	53 (48)	NR	NR
Summary	757	21	239 (36)	10	119 (18)
Current study (SCC)		27		15	
Current study (NECC)		51		44	

Cross-talk between neuroendocrine and immune network

TNF α induces neuroendocrine differentiation in small cell lung cancer cell lines


Haley et al, Am J Physiol Lung Cell Mol Physiol, 1998

TNF α Promoter Single Nucleotide Polymorphisms in Gastroenteropancreatic Neuroendocrine Tumors

Berkovic et al, Neuroendocrinology, 2006

TNF α persistently activates NF- κ B signaling in chromaffin Cells- implications for long-term regulation of neuropeptide gene expression in inflammation

Ait -Ali et al, Endocrinology, 2008

- 
- We presented a large series of 102 NECC, showing that
 - LCNEC and ATC were with worse prognosis.
 - Stage and tumor size were significant prognostic factors in SCNEC
 - ¼ of SCNEC were with long term survival
 - Standard adjuvant Tx to be established

 - Our data provide evidence for TNF α promotor polymorphisms over -308 and -1031 regions in neuroendocrine adenocarcinoma of cervix comparing to SCC.



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THANKS FOR ATTENTION!