INDOLEAMINE 2,3 DIOXYGENASE (IDO) EXPRESSION IN LUNG CANCER

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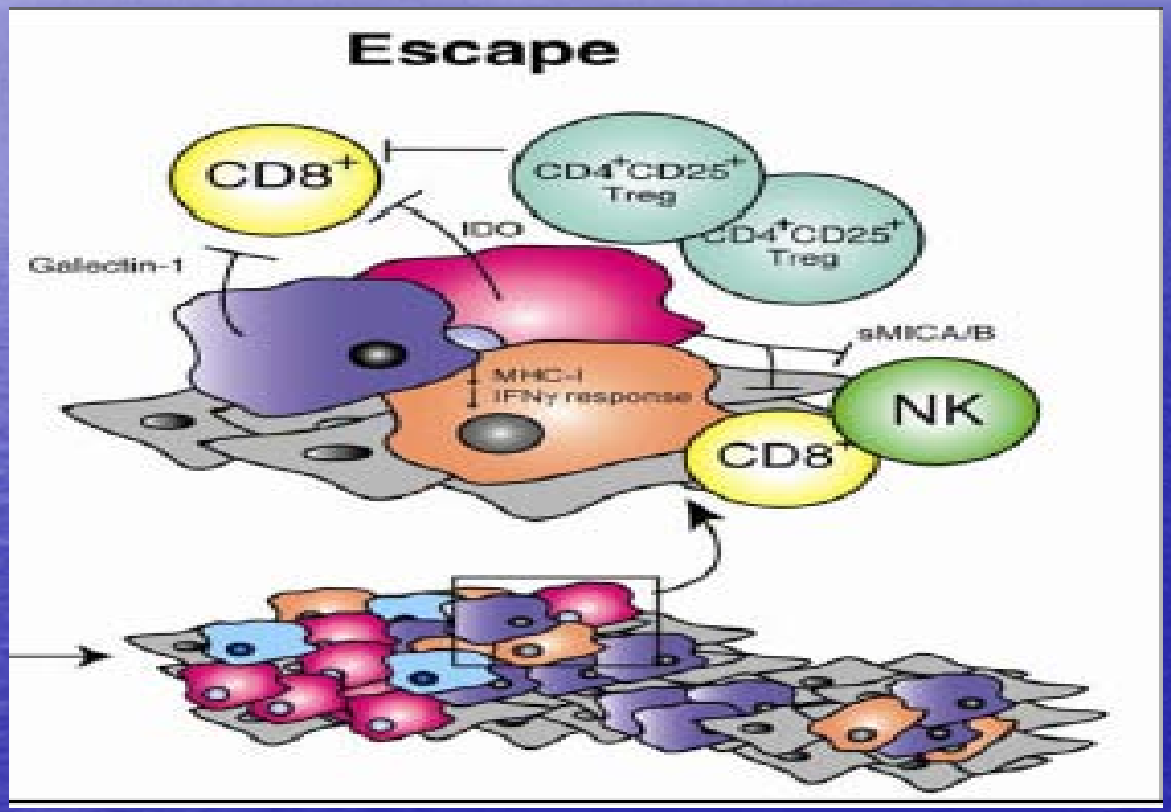
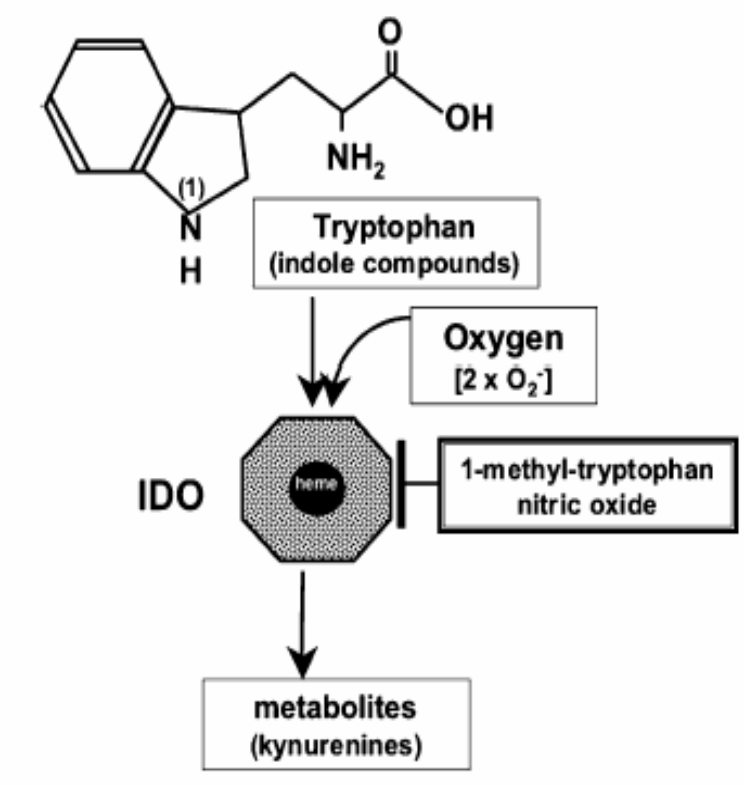
- Tumor –immune response
- Immune escape mechanisms



Munn H and Mellor L 2004; *Trends Mol. Med.* 10:15-18

Indoleamine 2,3 Dioxygenase - IDO

- L- tryptophan $\xrightarrow{\text{IDO}}$ N- formylkynurenine



- cytosolic monomeric hemoprotein
- 403aa long (MW 45,324KD)

Tone 1989

IDO functions

- antimicrobial defence mechanism
- maternal tolerance toward the allogeneic fetus
- suppresses T-cell responses to MHC-mismatched allografts and to autoantigens in animal models of disease
- role in immune escape of tumor cells

IDO expression

- Expressed constitutively at high levels in placenta, gut and epididymis, and at lower levels in spleen, lymph nodes and thymus

- in the lung IDO is expressed at basal levels

IDO and Lung cancer

most human tumors constitutively express IDO

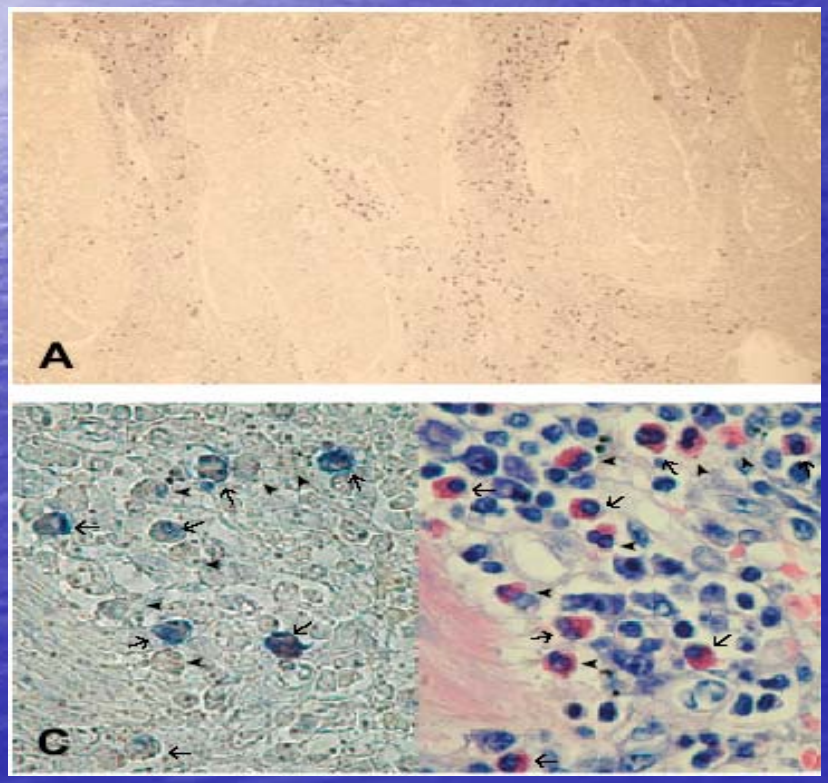
Tumor type	IDO-positive tumor samples[a] (no. positive per no. tested)
Prostatic carcinomas	11/11
Colorectal carcinomas	10/10
Pancreatic carcinomas	10/10
Cervical carcinomas	10/10
Endometrial carcinomas	5/5
Gastric carcinomas	9/10
Glioblastomas	9/10
Non-small-cell lung carcinomas	9/11
Bladder carcinomas	8/10
Ovarian carcinomas	8/10
Head and neck carcinomas	7/11
Esophageal carcinomas	7/10
Mesotheliomas	6/10
Renal cell carcinomas	5/10
Melanomas	11/25
Breast carcinomas	3/10
Thyroid carcinomas	2/10
Lymphomas	4/18
Small-cell lung carcinomas	2/10
Sarcomas	2/10
Hepatocarcinomas	2/5
Adrenal carcinomas	2/5
Choriocarcinomas	1/5
Cutaneous basocellular carcinomas	1/5
Testicular seminomas	0/10

Uyttenhove C 2003; *Nature Med.*

IDO and cancer

IDO is expressed by :

- Cancer cells themselves
- Cells in the infiltrating zone (macrophages, DCs)
- Cells in tumor-draining lymph nodes

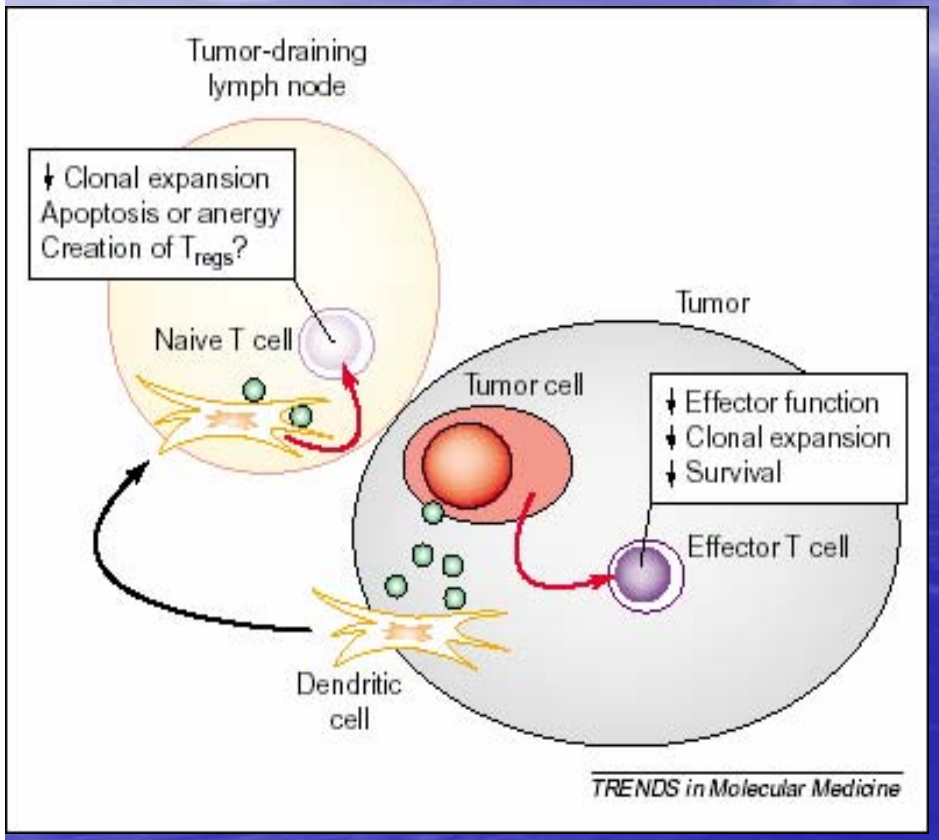


Subsets of cells express IDO in lung

eosinophil granulocytes (NSCLC)

Astigiano S et al 2005, Neoplasia

How does IDO promote immune escape



- Low tryptophan concentration
- Downstream metabolites (L-kynurenine, 3-hydroxyanthranilic acid)

Hwu P et al. 2000; J Immun 164:3596-99, Woo E et al 2001;Cancer Research 61:4766–72,
Mellor A et al. 2002; J. Immun. 168: 3771-76, Munn H and Mellor L 2004; *Trends Mol. Med.* 10:15-18

AIM:

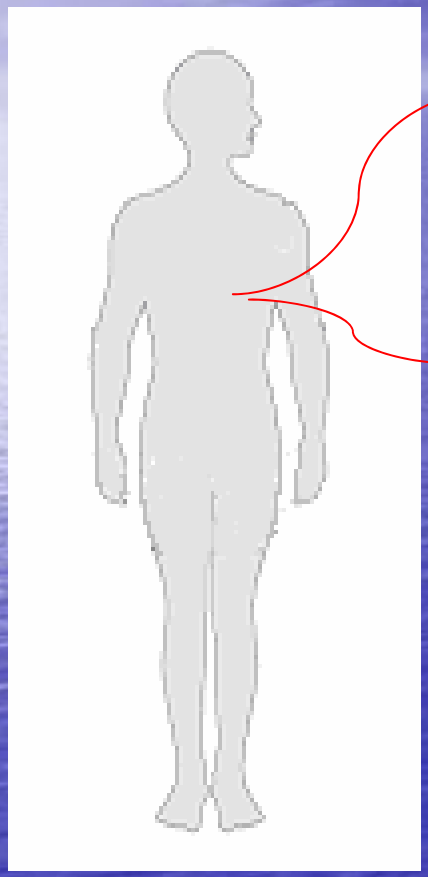
- To investigate the expression of IDO
  - in lung cancer cell lines
  - surgically resected lung cancer tissues
  - autologous non malignant samples

- Correlations of IDO expression with clinicopathological parameters

Material

Patient no	Gender	Age	Histology	Differentiation	pTNM status	pStage	Tumour volume (mm^3)
1	F	56	ADC	poor	T1N1M0	IIA	15
2	M	65	ADC	moderate	T3N0M0	IIB	56
3	M	65	ADC	poor	T1N2M0	IIIA	15
4	F	48	ADC	moderate	T1N0M0	IA	27
5	F	59	ADC	well	T2N2M0	IIIA	120
6	M	73	ADC	moderate	T2N0M0	IB	52
7	M	55	ADC	moderate	T4N2M0	IIIB	57
8	M	60	ADC	poor	T2N1M0	IIB	35
9	M	65	ADC	poor	T3N0M0	IIB	55
10	M	74	ADC	poor	T2N0M0	IB	26
11	M	48	ADC	poor	T3N1M0	IIIA	1188
12	M	71	ADC	poor	T2N2M0	IIIA	361
13	M	61	SCC	poor	T2N0M1	IV	54
14	M	67	SCC	moderate	T1N0M0	IA	8
15	M	55	SCC	poor	T2N1M0	IIB	42
16	M	79	SCC	moderate	T2N0M0	IB	14
17	M	70	SCC	moderate	T2N0M0	IB	12
18	M	63	SCC	moderate	T3N1M0	IIIA	27
19	M	79	SCC	poor	T2N0M0	IB	37
20	M	59	SCC	moderate	T2N1M0	IIB	23
21	M	62	SCC	poor	T2N0M0	IB	14
22	M	59	SCC	moderate	T1N1M0	IIA	13
23	M	58	SCC	poor	T2N0M0	IB	29
24	F	76	SCC	moderate	T2N0M0	IB	428
25	M	40	SCLC	poor	T3N1M0	IIIA	225
26	F	75	BAC	moderate	T2N0M0	IB	120
27	M	74	BAC	moderate	T2N1M0	IIB	150
28	M	59	BAC	poor	T4N0M1	IV	31

Methods



tumor tissue

normal tissue
2.5 - 11 cm

cancer cell lines:
CALU-1, CALU-6, GILI, ONET, SK-LU-1, NCI-H441, NCI-H460, NCI-H596, NCI-H661

Reference tissue
(amartoma)

RNA extraction

cDNA synthesis

qReal Time PCR for ABL and IDO

Methods

- **qReal Time –PCR**

SYBR supermix kit (Invitrogen, Paisley UK)

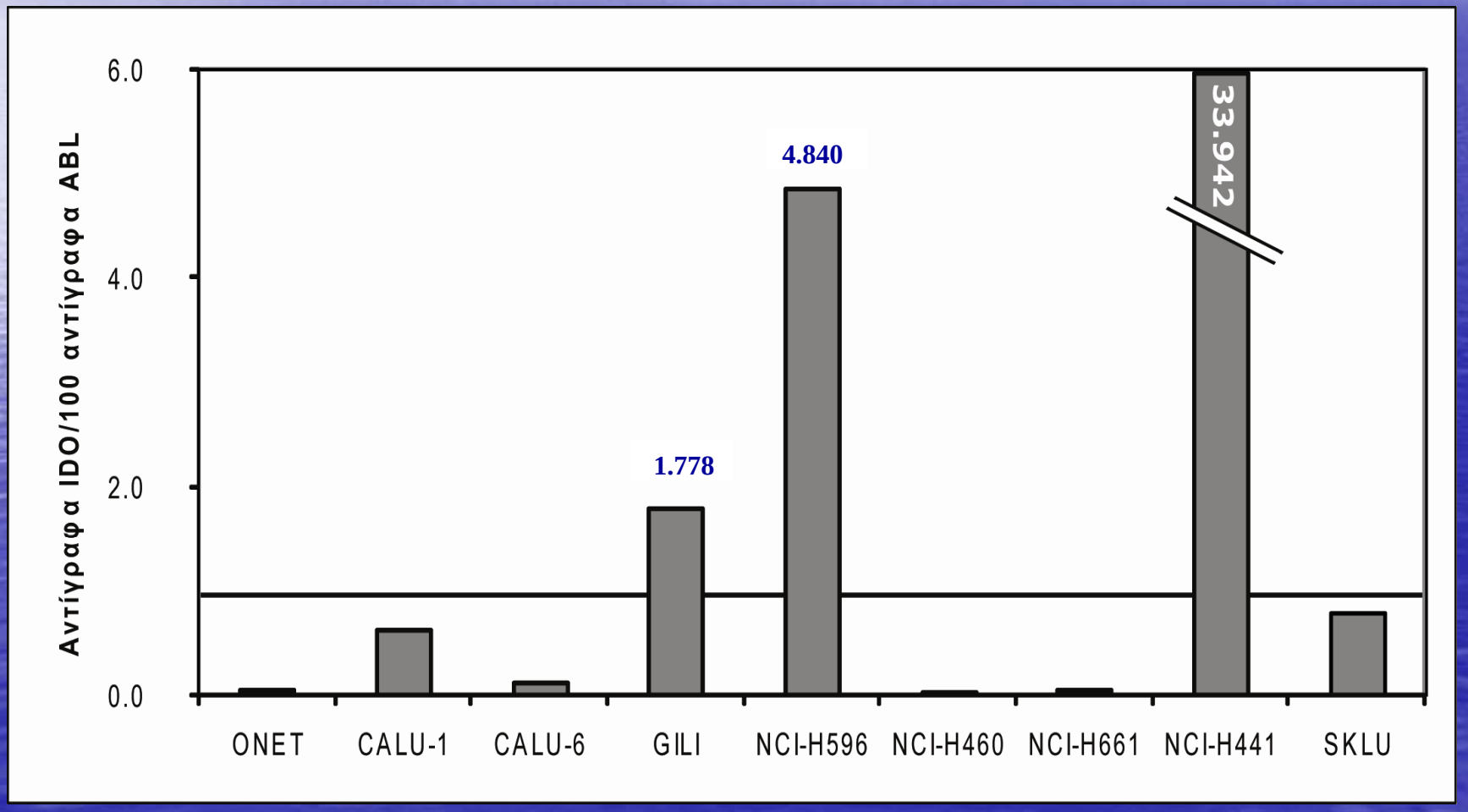
FORWARD 5' GGTCATGGAGATGTCCGTAA 3'
REVERSE 5' ACCAATAGAGAGACCAGGAAGAA 3'
50°C 5 s, 95°C 10 min and then
45 cycles of 95°C 15 s and 60°C 1min.

- Rotor Gene software

Results

IDO –LC Cell lines 3 / 9

4.7 ± 11.1 (0.0-33.9)



Results

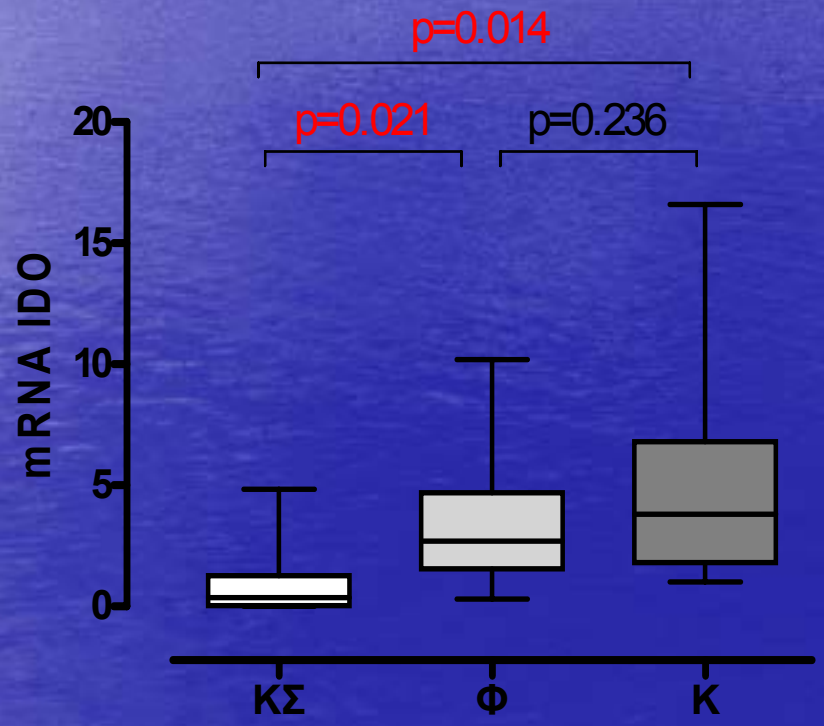
21 / 24 Tumor

23 / 27 Normal

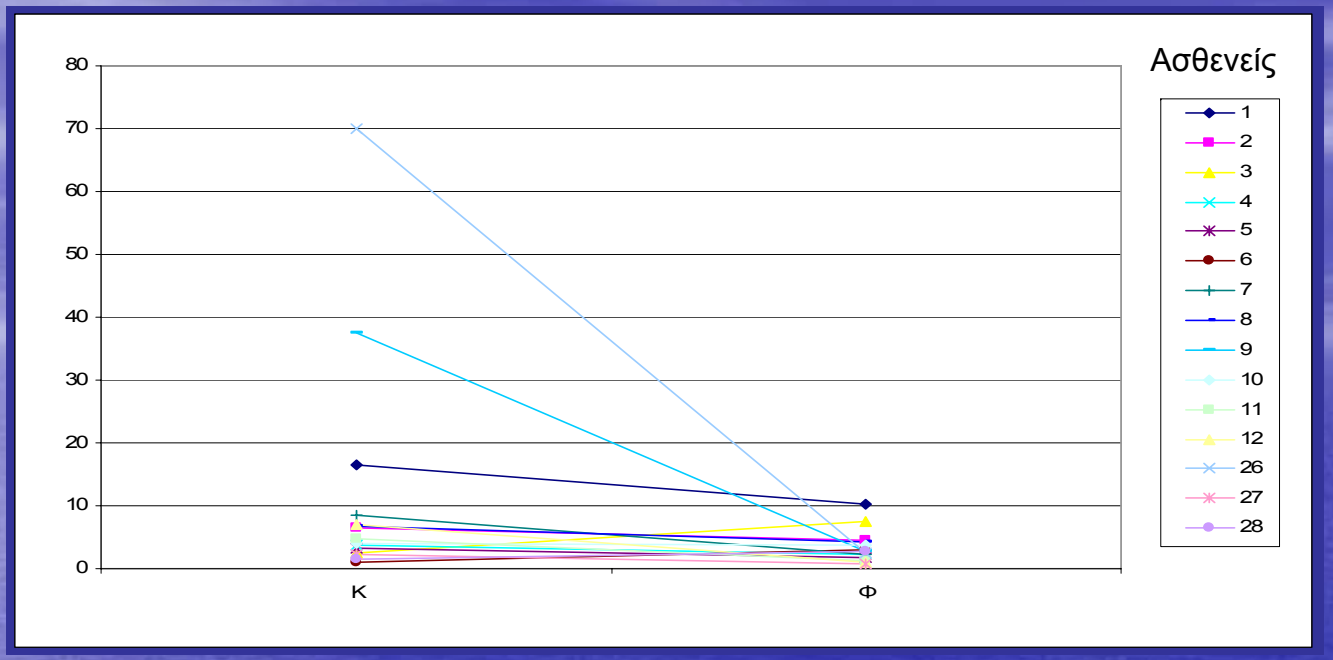
Patient no	IDO Expression by Tumor[a]	IDO Expression by Normal[a]	Ratio T/N
1	16.6	10.2	1,6
2	6.5	4.4	1,5
3	2.6	7.5	0,3
4	3.8	2.3	1,6
5	3.3	1.7	1,9
6	1.0	3.0	0,3
7	8.5	2.1	3,9
8	6.8	4.1	1,6
9	37.5	2.7	14,1
10	3.9	3.8	1,0
11	4.7	1.2	3,8
12	7.1	0.9	7,5
13	6.6	4.5	1,5
14	1.8	0.3	6,4
15	1.0	N/A[b]	-
16	1.0	1.4	0,7
17	N/A	8.8	-
18	9.0	4.9	1,8
19	2.9	2.2	1,3
20	N/A	5.2	-
21	N/A	N/A	-
22	N/A	N/A	-
23	1.9	0.6	3,2
24	1.6	2.0	0,8
25	1.1	2.2	0,5
26	70.0	2.4	29,1
27	2.4	0.8	3,1
28	1.5	2.7	0,5

a **copies IDO/100 copies ABL** . b Not available.

The relative expression of IDO in lung cancer cell lines (4.7±11.1) was significantly lower than that of all patients' tumor samples as well as that of the autologous non affected lung tissues



Adeno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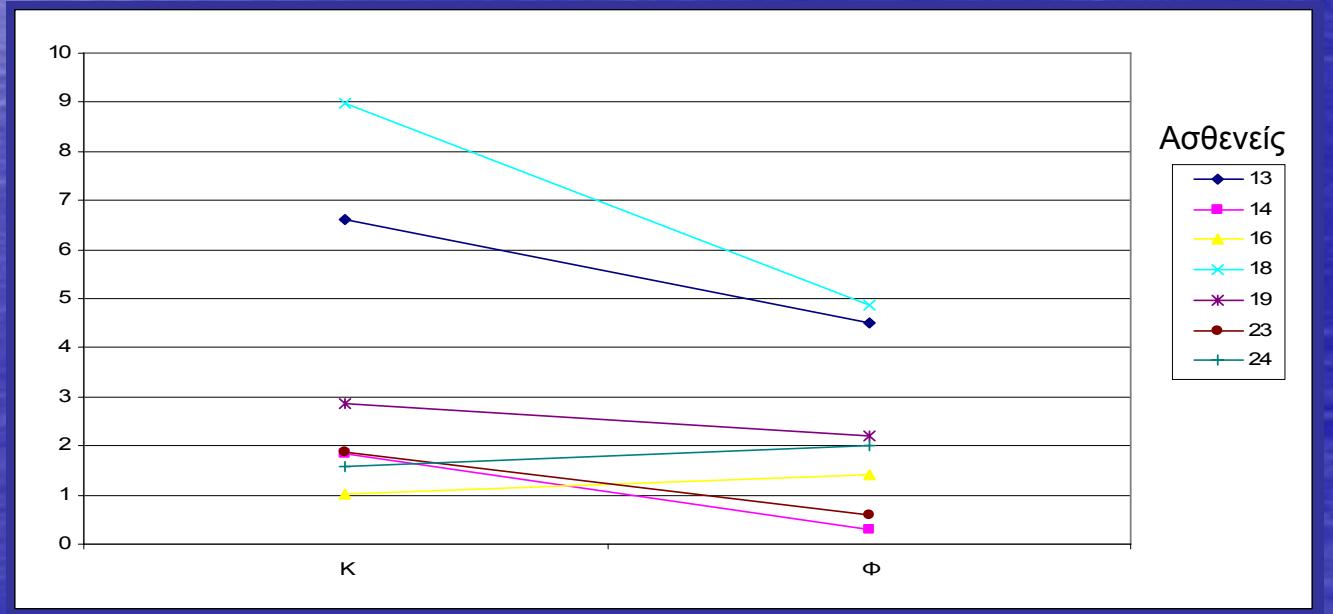
SCC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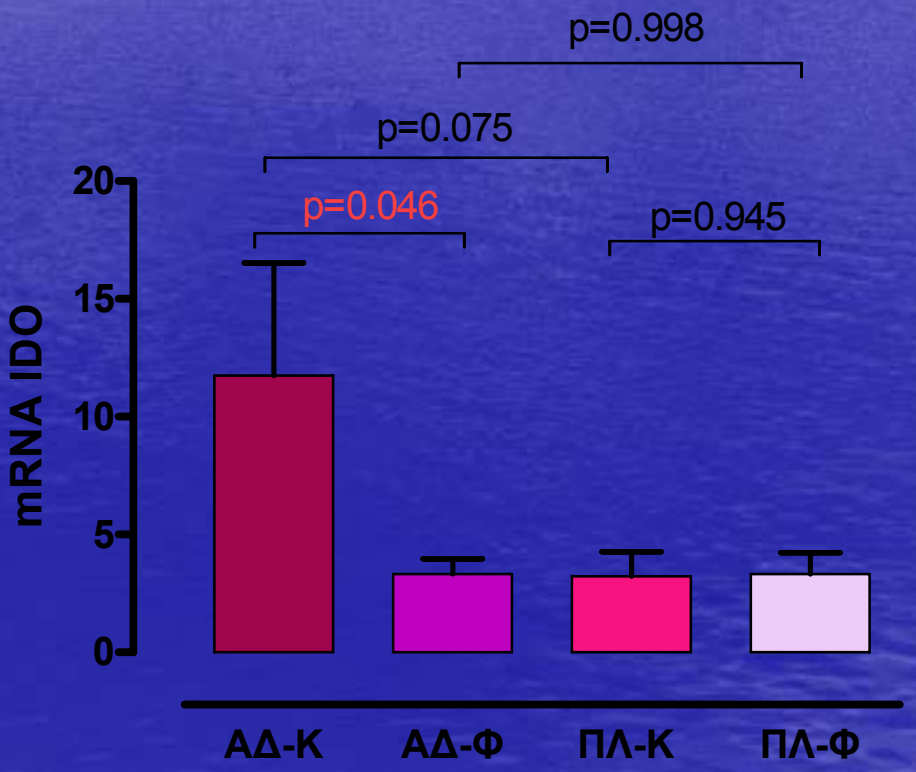
IDO		
	Tumor	Normal
Patients	8.46 ± 15.18	3.28 ± 2.51
Adeno	11.75 ± 18.52	3.32 ± 2.54
SCC	3.22 ± 2.96	3.32 ± 2.75

Only in ADC the relative expression of IDO was higher in tumor samples than in non malignant lung tissues.

No statistically significant differences were noted between ADC and SCC regarding either the tumor samples or the autologous non affected samples.



No significant correlations between IDO expression and clinicopathological parameters were found

				p-value
Age	<70	16	7.07 ± 9.06	0.538
	≥70	8	11.24 ± 23.83	
SEX	♂	19	5.67 ± 8.17	0.078
	♀	5	19.06 ± 29.10	
Smoke history	Non smokers	4	12.55 ± 16.73	0.590
	Ex smokers	7	5.18 ± 5.64	
	Smokers	13	8.96 ± 18.53	
Type	Adeno	15	11.75 ± 18.52	0.075
	SCC	8	3.22 ± 2.96	
Differentiation	G1-G2	11	9.90 ± 20.14	0.772
	G3	13	7.24 ± 10.00	
T	T1-T2	17	7.90 ± 16.45	0.309
	T3-T4	7	9.82 ± 12.59	
N	N0	13	10.77 ± 20.28	0.417
	N1-N2	11	5.73 ± 4.59	
M	M0	22	8.86 ± 15.80	
	M1	2	4.05 ± 3.60	
Stage	I–II	15	10.58 ± 18.99	0.551
	III-IV	9	4.93 ± 2.98	
Tumor volume	<50 mm^3	12	4.40 ± 4.53	0.340
	≥50 mm^3	12	12.53 ± 20.62	

Conclusion:

- Direct evidence is provided demonstrating that IDO mRNA can be constitutively expressed by lung cancer cells.

- The higher IDO expression observed in patients' samples can be attributed to the production of the enzyme by other cells recruited in the tumor microenvironment and the peri-tumoral lung area and/or to its induction by soluble factors of tumor origin.

- Future : IDO inhibition

(immunotherapy and chemotherapy protocols)

Collaborators:

Department of Immunology and Histocombatibility, UHL

Department of Pathology, UHL

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Thoracic Surgery Department, Athens Medical Center

Ludwig Institute for Cancer Research, Belgium

Austin Research Institute, Australia

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THANKS FOR YOUR ATTENTION