6th YSS

Future-proofing Bioanalysis - Contributing to a sustainable world

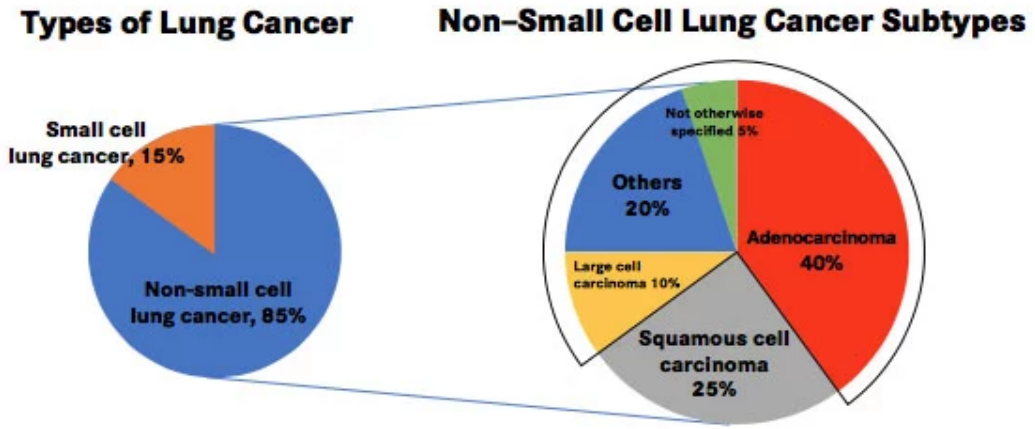
Indoleamine 2,3-dioxygenase 2 as a new biomarker of tumor progression

Mondanelli Giada

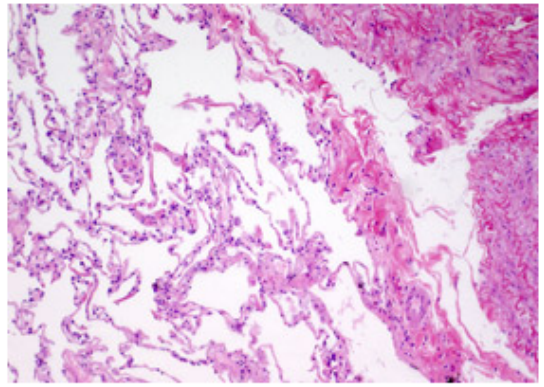
Lung cancer is one of the major cause of cancer-related morbidity and mortality across the globe

FIGURE 1. Types of Lung Cancer by Histology[1,4,5]

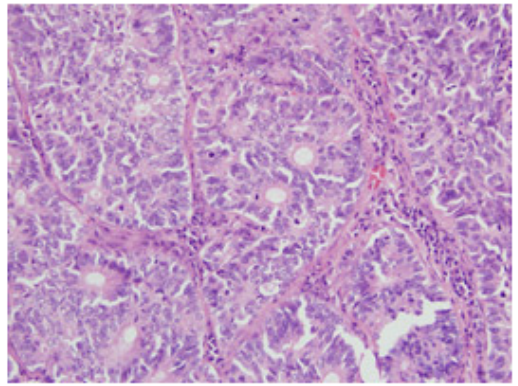
Types of Lung Cancer	
Non-small cell lung cancer	85%
Small cell lung cancer	15%
Non-Small Cell Lung Cancer Subtypes	
Adenocarcinoma	40%
Squamous cell carcinoma	25%
Large cell carcinoma	25%
Others	20%
Not otherwise specified	5%



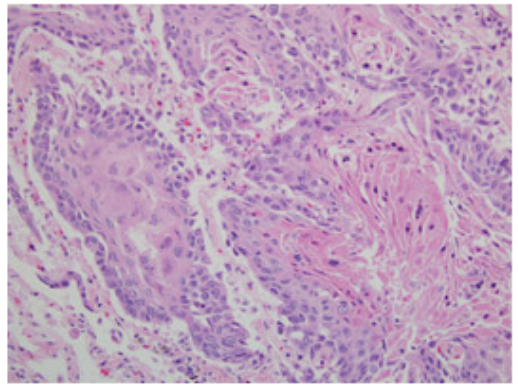
SEER stage	5-year relative survival rate
Localized	61%
Regional	35%
Distant	6%



Normal lung tissue



Lung - adenocarcinoma



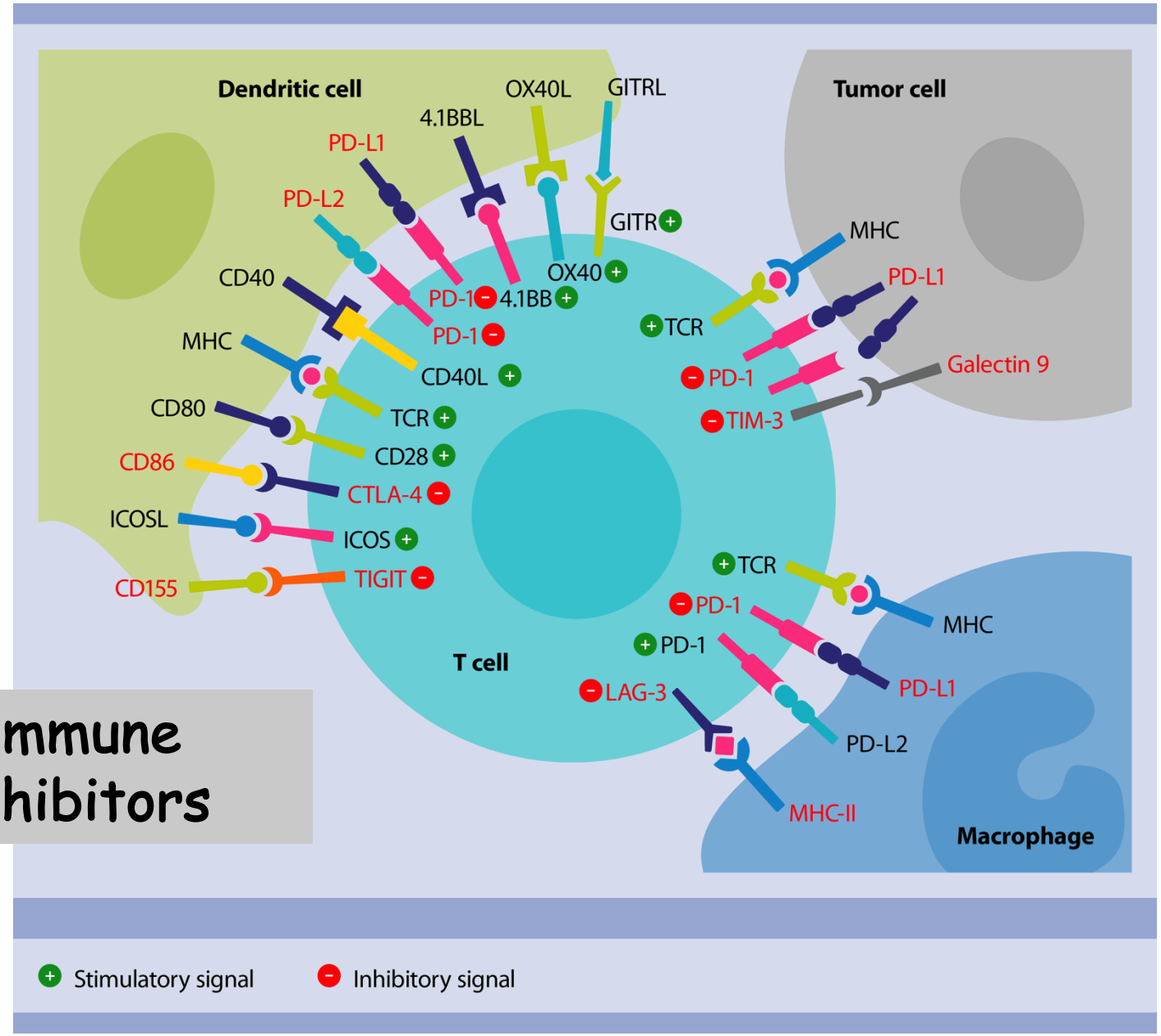
Lung - squamous cell carcinoma

Source: American Cancer Society

Immune checkpoints are regulators of the immune responses

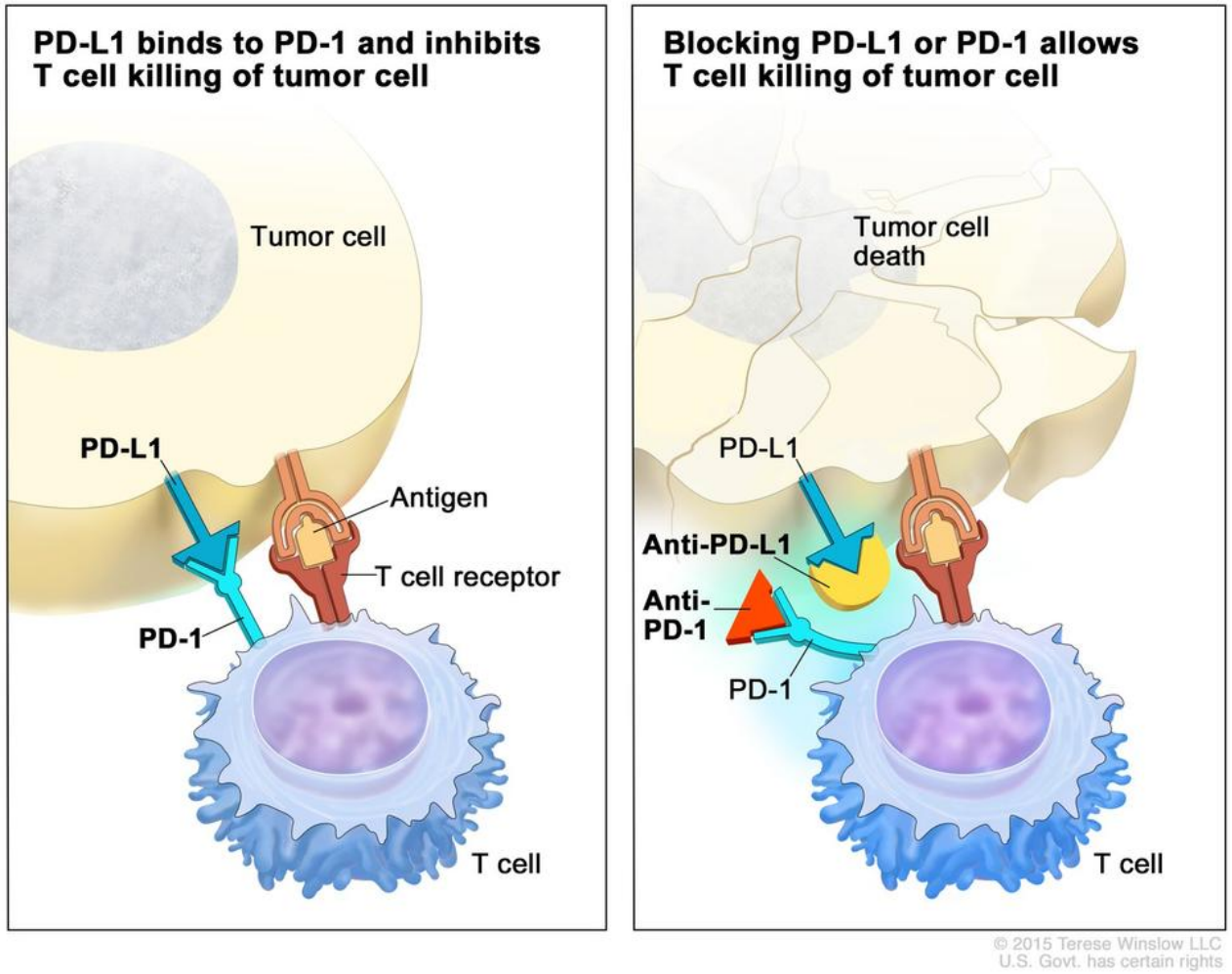
Homeostasis

Tumor immune escape



Targets of immune checkpoint inhibitors

Immune checkpoints inhibitors for the treatment of NSCLC



Nivolumab (OPDIVO®) and Pembrolizumab (KEYTRUDA ®) are monoclonal antibodies specific for PD-1

Atezolizumab (TECENTRIQ ®) is a monoclonal antibody specific for PD-L-1

FDA-approved drugs for the treatment of advanced pretreated NSCLC

Table 1 Clinical trials on immune checkpoint inhibitors in non–small cell lung cancer

Name of trial	Phase	Histology/ line of treatment	Randomization	No. Cases	First end point results	ORR (RECIST)	Effect of PD-L1 expression
CheckMate 017	III	SqNSCLC/ second	Nivolumab at 3 mg/kg vs. docetaxel at 75 mg/m^2	272	Significant improvement in OS for patients receiving nivolumab compared with docetaxel (median, 9.2 vs. 6.0 mo; HR, 0.59; $p < .001$).	Response rate was 20% with nivolumab vs. 9% with docetaxel ($P = 0.008$)	PD-L1 expression was neither prognostic nor predictive for efficacy end points
CheckMate 057	III	Non-SqNSCLC/ second	Nivolumab at 3 mg/kg vs. docetaxel at 75 mg/m^2	582	Significant improvement in OS for patients receiving nivolumab compared with docetaxel (median 12.2 vs. 9.4 mo; HR, 0.73; $p = .002$).	Response rate was 19% with nivolumab vs. 12% with docetaxel ($P = 0.02$)	PD-L1 expression was associated with even greater efficacy at all expression levels (≥1%, ≥5%, and ≥ 10%).
KEYNOTE 010	II/III	NSCLC PD-L1-positive tumors (PS ≥ 1%)/second	Pembrolizumab at 2 mg/kg or 10 mg/kg vs. docetaxel 75 mg/m^2	1034	Significant improvement in OS for pembrolizumab at 2 mg/kg (median 10.4 vs. 8.5 mo; HR, 0.71; $p = .0008$) and pembrolizumab at 10 mg/kg (median, 12.7 vs. 8.5 mo; HR, 0.61; $p < .001$) compared with docetaxel	Response rate was 18% with pembrolizumab (2 mg and 10 mg vs. 9% with docetaxel ($P = 0.0005$ and 0.0002)	Pembrolizumab efficacy was greater in patients with tumor PS ≥50%
KEYNOTE 024	III	NSCLC, PD-L1-positive tumors (PS ≥50%), no sensitizing mutation of EGFR or translocation of ALK/first	Pembrolizumab at fixed dose of 200 mg or platinum-based chemotherapy	305	Significant improvement in PFS for patients receiving pembrolizumab compared with chemotherapy (median 10.3 vs. 6.0 mo; HR, 0.5; $p < .00001$).	Response rate was 44.8% with pembrolizumab vs. 27.8% with chemotherapy	All patients, PD-L1 expression on at least 50% of tumor cells
POPLAR	II	NSCLC/ second	Atezolizumab 1200 mg vs. docetaxel 75 mg/m^2	287	Significant improvement in OS for patients receiving atezolizumab compared with docetaxel (median, 12.6 vs. 9.7 mo; HR, 0.73; $P = .04$)	Objective responses with atezolizumab were durable, with a median duration of 14·3 months (95% CI 11·6–non-estimable) compared with 7·2 months (5·6–12·5) for docetaxel	As with OS, PFS and ORR tended to show increased atezolizumab benefit with increasing PD-L1 expression.
OAK	III	NSCLC/ second	Atezolizumab at 1200 mg vs. docetaxelat 75 mg/m^2	850	Significant improvement in OS for patients receiving atezolizumab compared with docetaxel (median 13.8 vs. 9.6 mo; HR, 0.73; $P = .0003$).	For ITT population, response rate was 14% with atezolizumab vs. 13% with docetaxel	Overall survival was improved regardless of PD-L1 expression levels. Patients with tumors expressing high levels of PD-L1 (TC3 or IC3) derived the greatest benefit from atezolizumab
PACIFIC	III	Stage III NSCLC with no disease progression after ≥2 cycles of chemoradiotherapy/ second	Durvalumab at 10 mg/kg vs. placebo	709	Significant improvement in PFS and OS for patients with durvalumab vs. with placebo (PFS median 17.2 vs. 5.6 mo; HR, 0.51, $P < 0.001$; HR for OS =0.68, $P = 0,0025$);	Response rate was 28% with durvalumab vs. 16% with placebo	PFS and OS benefits with durvalumab were observed in all subgroups, including PD-L1 expression ≥25% or < 25%

Abbreviations: *OS* overall survival, *NSCLC* non–small cell lung cancer, *Sq* squamous, *HR* hazard ratio, *ORR* objective response rate, *ITT* Intent to treat, *PD-1*, programmed cell death protein-1, *PD-L1* programmed cell death ligand-1, *PS* proportion score

PD-L-1 expression as a prognostic or predictive biomarker

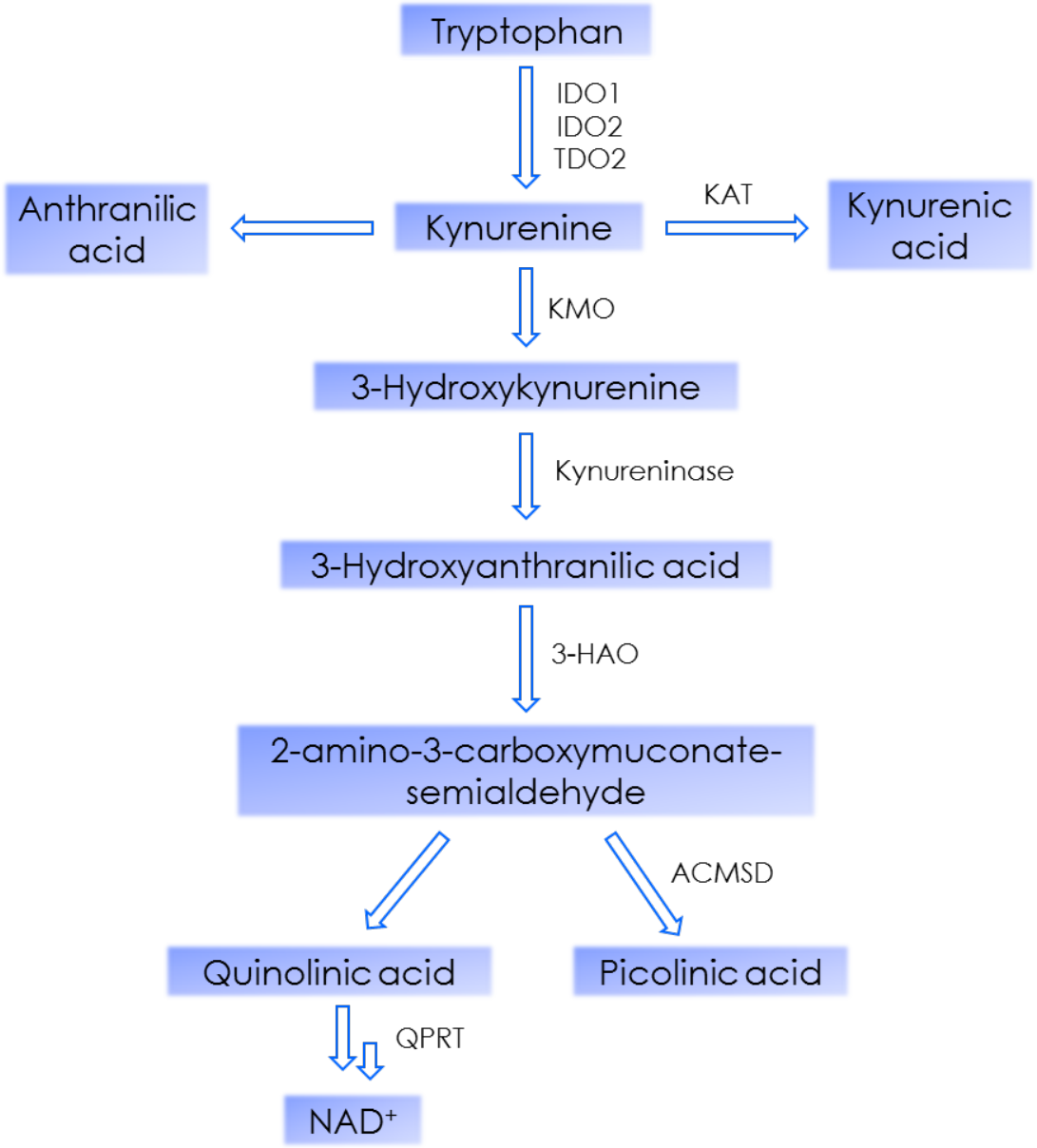
Nivolumab was found to be significantly better than docetaxel regardless of intratumoral PD-L1 expression levels.

Pembrolizumab efficacy was greater in patients with PD-L-1 expression ≥ 1% (second-line therapy) or ≥50% (first-line therapy)

- Heterogeneous expression of PD-L-1
- Differences between primary tumor and metastatic lesions

Predictive biomarkers associated with response to immune checkpoint inhibitors

Tryptophan degrading enzymes as immunological control nodes

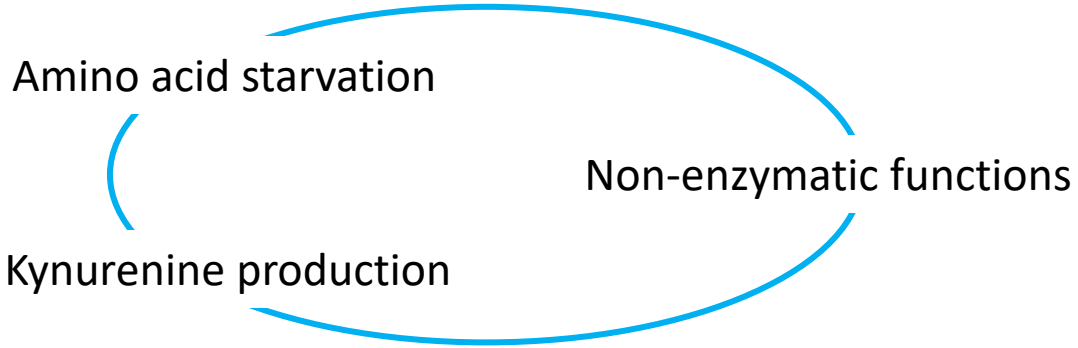


TDO, tryptophan 2,3-dioxygenase

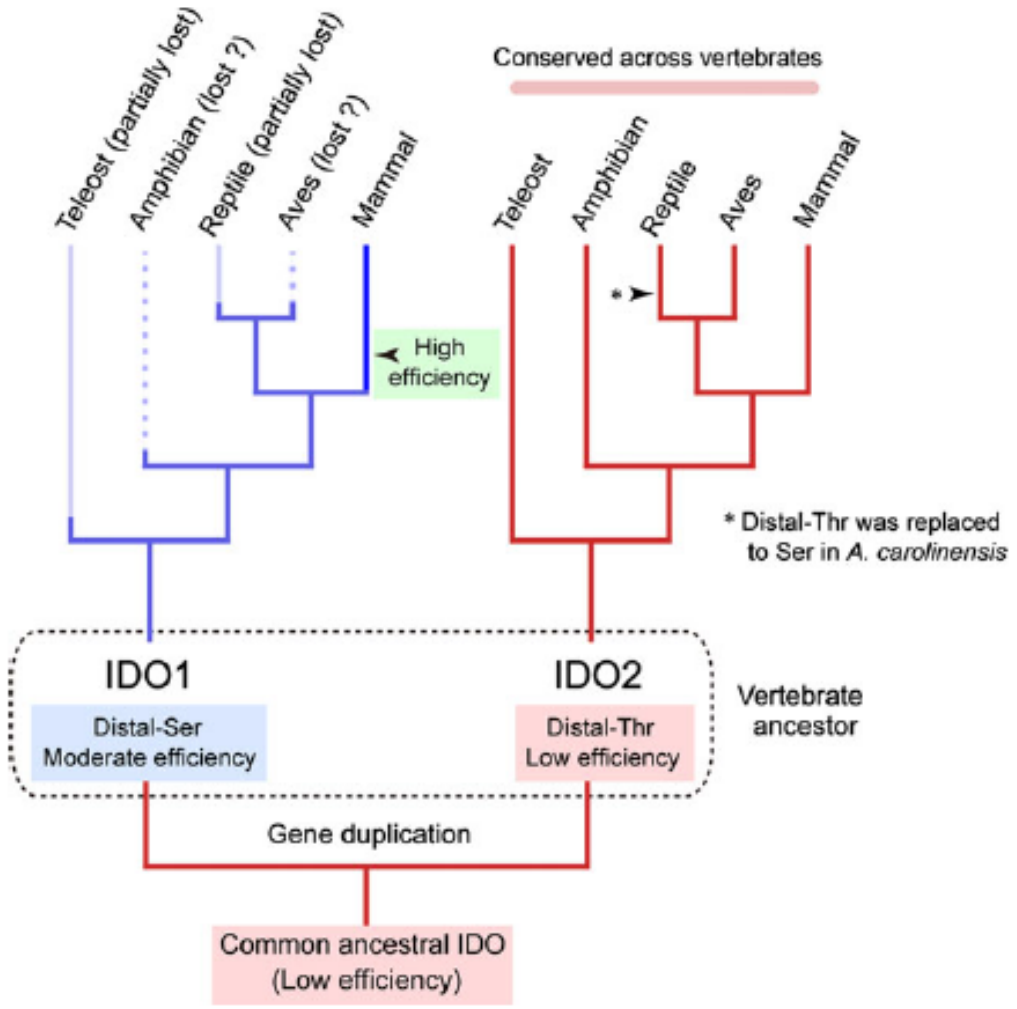
TDO has a narrower expression and substrate specificity

IDO1, indoleamine 2,3-dioxygenase 1

IDO1 enzyme is expressed in various tissues and can catabolize a wider range of indole-containing substrates



IDO1 and IDO2: the product of an ancient gene duplication event that occurred prior to the evolution of vertebrates



IDO2

- Is present in mammals and lower vertebrates
- has a low affinity for the substrate Trp

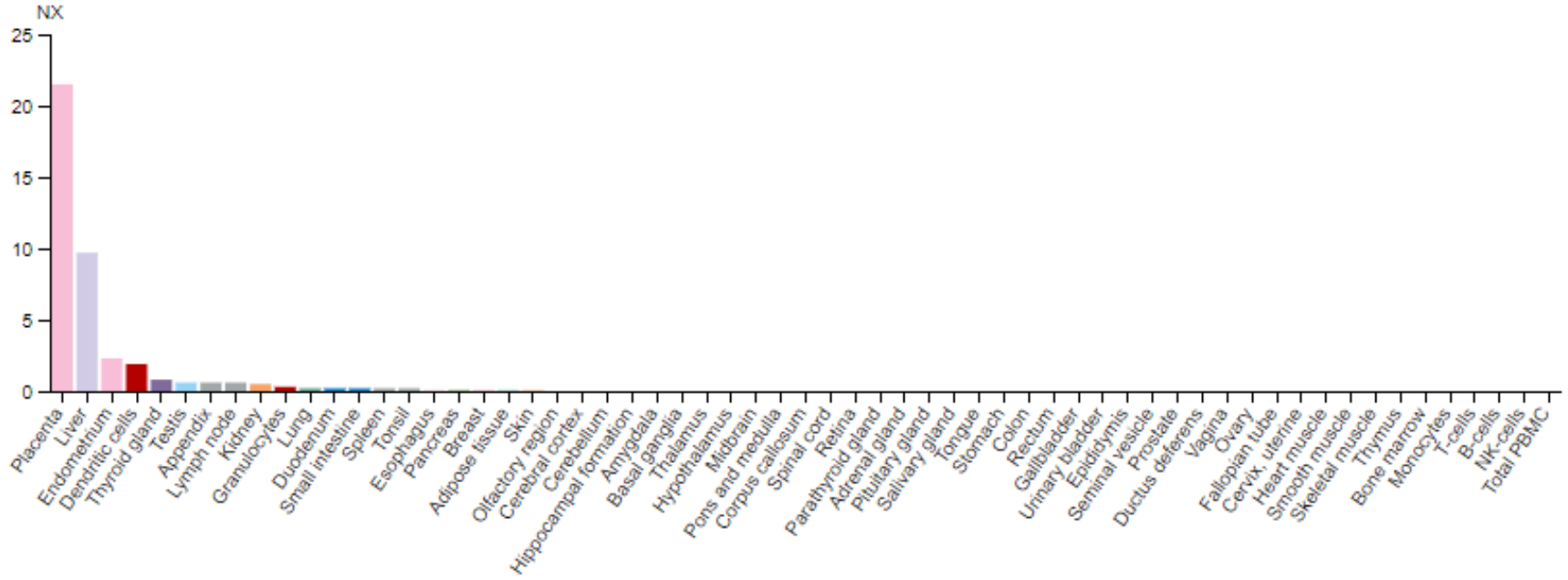
IDO1

has an high affinity for the substrate

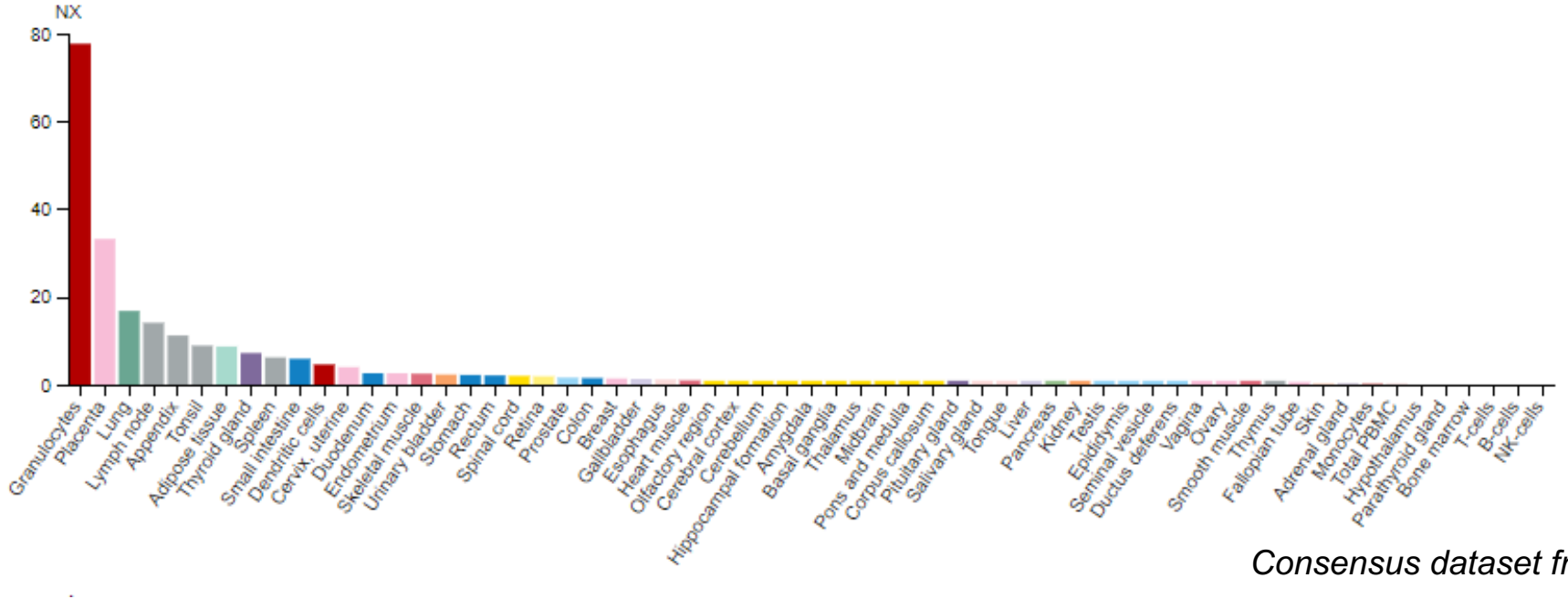
Yuasa HJ, et al. FEBS J. 2015;282(14):2735-2745.

Different expression of IDO1 and IDO2 in human tissues

IDO2

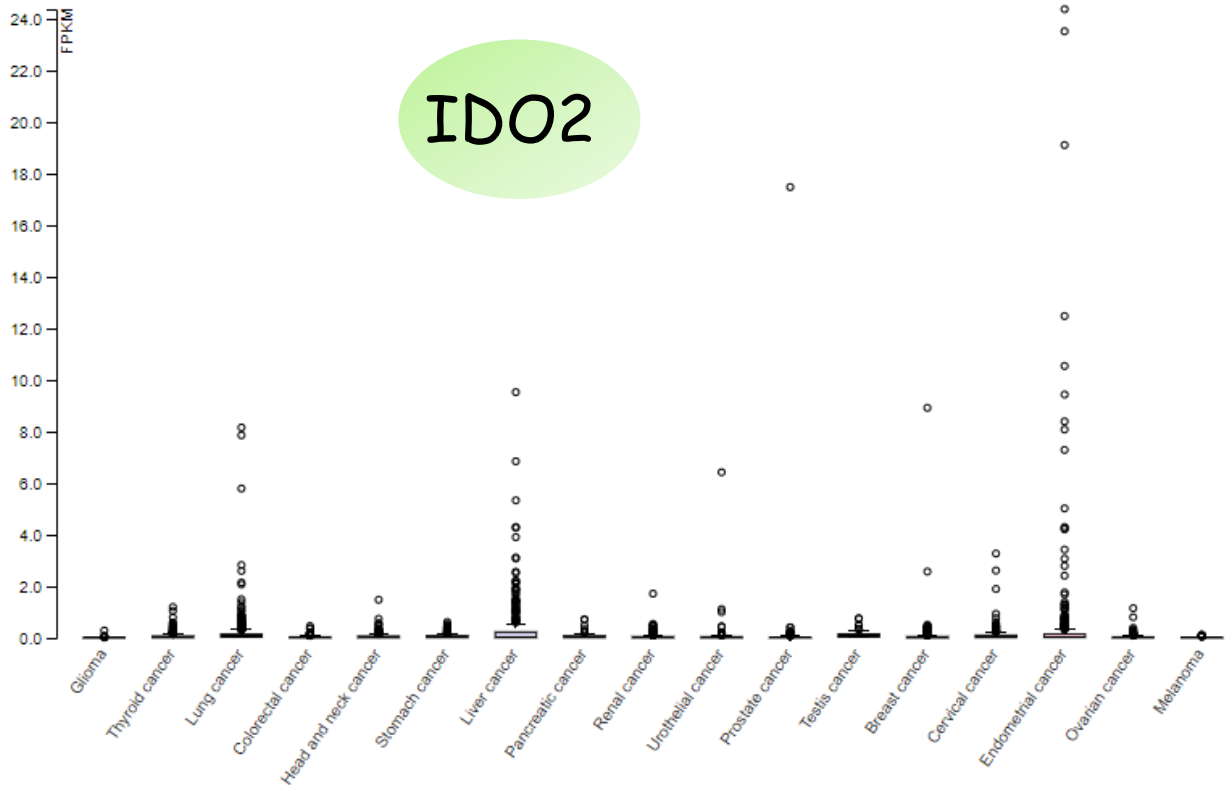


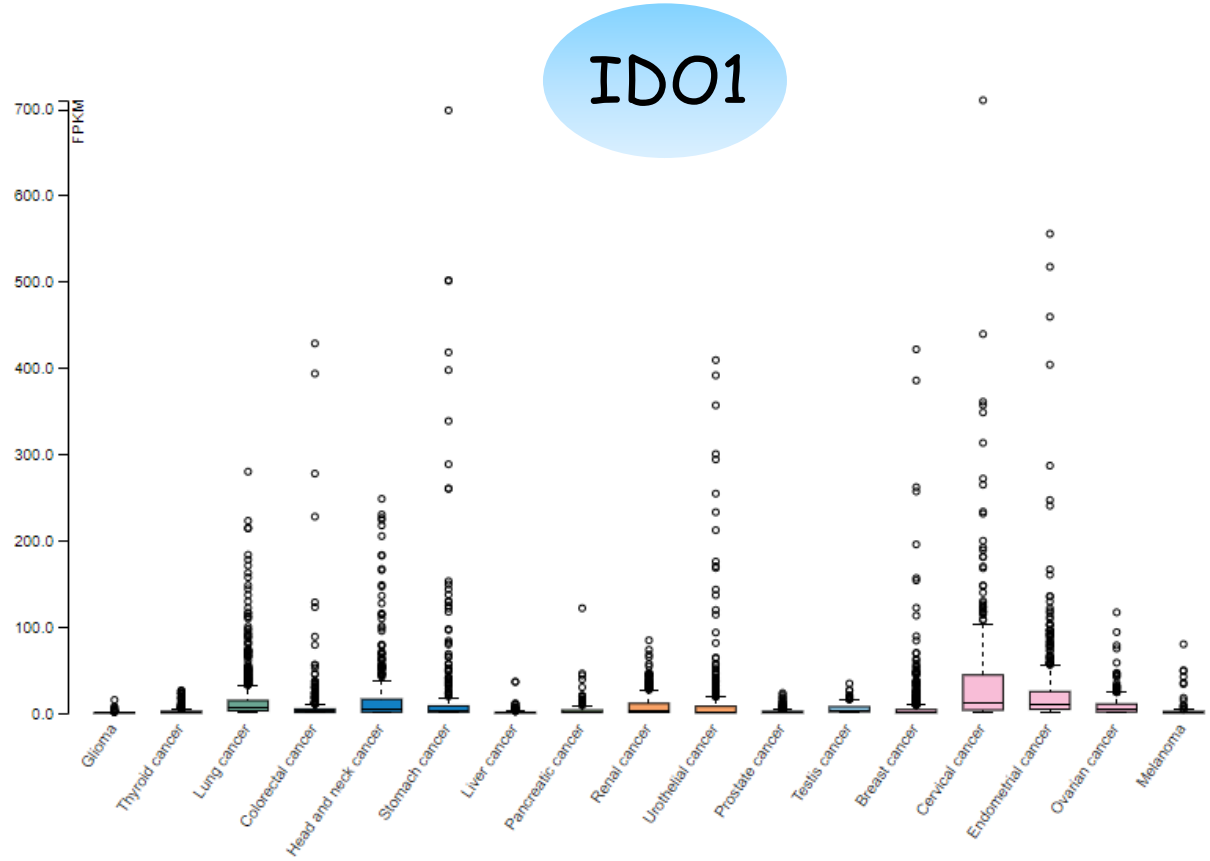
IDO1



Consensus dataset from Human Protein Atlas

Different expression of IDO1 and IDO2 in tumor tissues

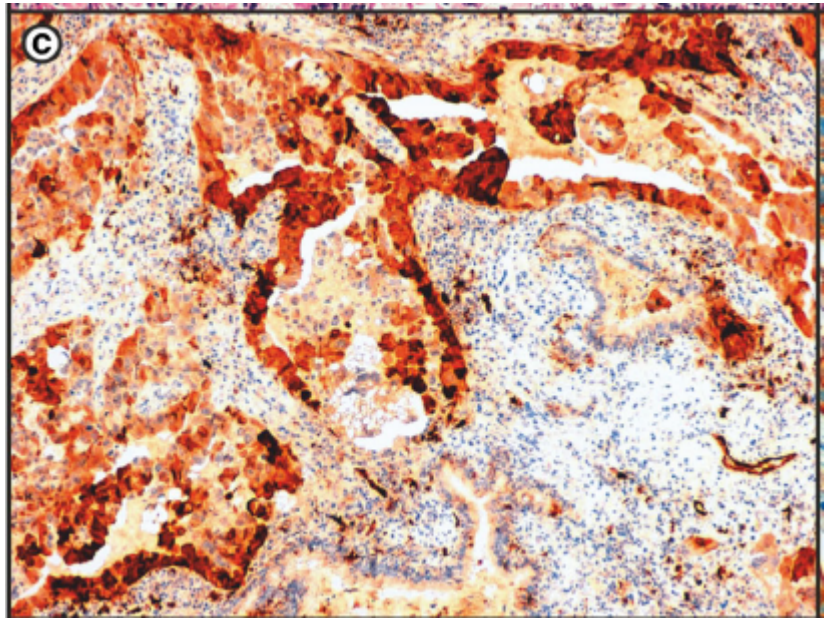




RNA-seq data from Human Protein Atlas

IDO1 is commonly expressed by NSCLC

Parameter	Adenocarcinomas (n. 122)						Squamous cell carcinomas (n. 69)					
		IDO-1 low		IDO-1 high				IDO-1 low		IDO-1 high		
		n. 56		n. 66				n. 31		n. 38		
		N	%	*N*	%	*p*		*N*	%	*N*	%	*p*
PD-L1 low	n. 100	51	51.00	49	49.00	*0.015*	n. 48	27	56.25	21	43.75	*0.004*
PD-L1 high	n. 22	5	22.73	17	77.27		n. 21	4	19.05	17	80.95	



Mandarano M, et al. Virchows Arch. 2019

What about IDO2?

- IDO2 expression in resected NSCLCs
- Correlation between IDO2 expression, clinical parameters and patients' prognosis

IDO2 as potential biomarker for NSCLCs

Parameter	IDO2 low		IDO2 high			Total	
	N	%	*N*	%	*p*	*N*	%
	31	**16**	**160**	**84**		**191**	**100**
GENDER							
M	23	17	114	83	0.739	137	72
F	8	15	46	85		54	28
AGE							
<68 years	13	15	74	85	0.659	87	46
≥68 years	18	17	86	83		104	54
SMOKING							
Current smokers	13	17	64	83	0.910	77	40
Former smokers	16	16	82	84		98	51
Never smokers	2	12	14	88		16	9
RELAPSE							
Yes	12	16	61	84	0.951	73	38
No	19	16	99	84		118	62
EXITUS							
Yes	6	9	58	91	0.068	64	34
No	25	20	102	80		127	66
STAGE							
Adc[a] stage						***122***	***64***
I	6	8	68	92	0.964	74	61
II - III	4	8	44	92		48	39
Sqcc[b] stage						***69***	***36***
I	7	26	20	74	0.513	27	39
II - III	14	33	28	67		42	61
HISTOTYPE							
Adc[a]	10	8	112	92	**<0.001**	122	64
Sqcc[b]	21	30	48	70		69	36
Adc[a] pattern						***122***	***64***
Other than solid	6	6	89	94	0.155	95	78
Solid	4	15	23	85		27	22

Patients cohort

The median age is 68 years
Median follow-up period of 50 months

53% stage I
47% stage II-III

34% died from NSCLC

64% adenocarcinomas
36% squamous cell carcinomas

The majority of NSCLC cases expresses high levels of IDO2

Staining score
- Sum of the intensity of the staining:
***0**: absent; **1+**: mild; **2+**: moderate; **3+**: intense*
- Percentage of tumor cells labeled:
***0**: 0%; **1**: 1–25%; **2**: 26–50%; **3**: 51–75%; **4**: 76–100%*

IDO2 low: 0 - 2

IDO2 high: 3 - 7

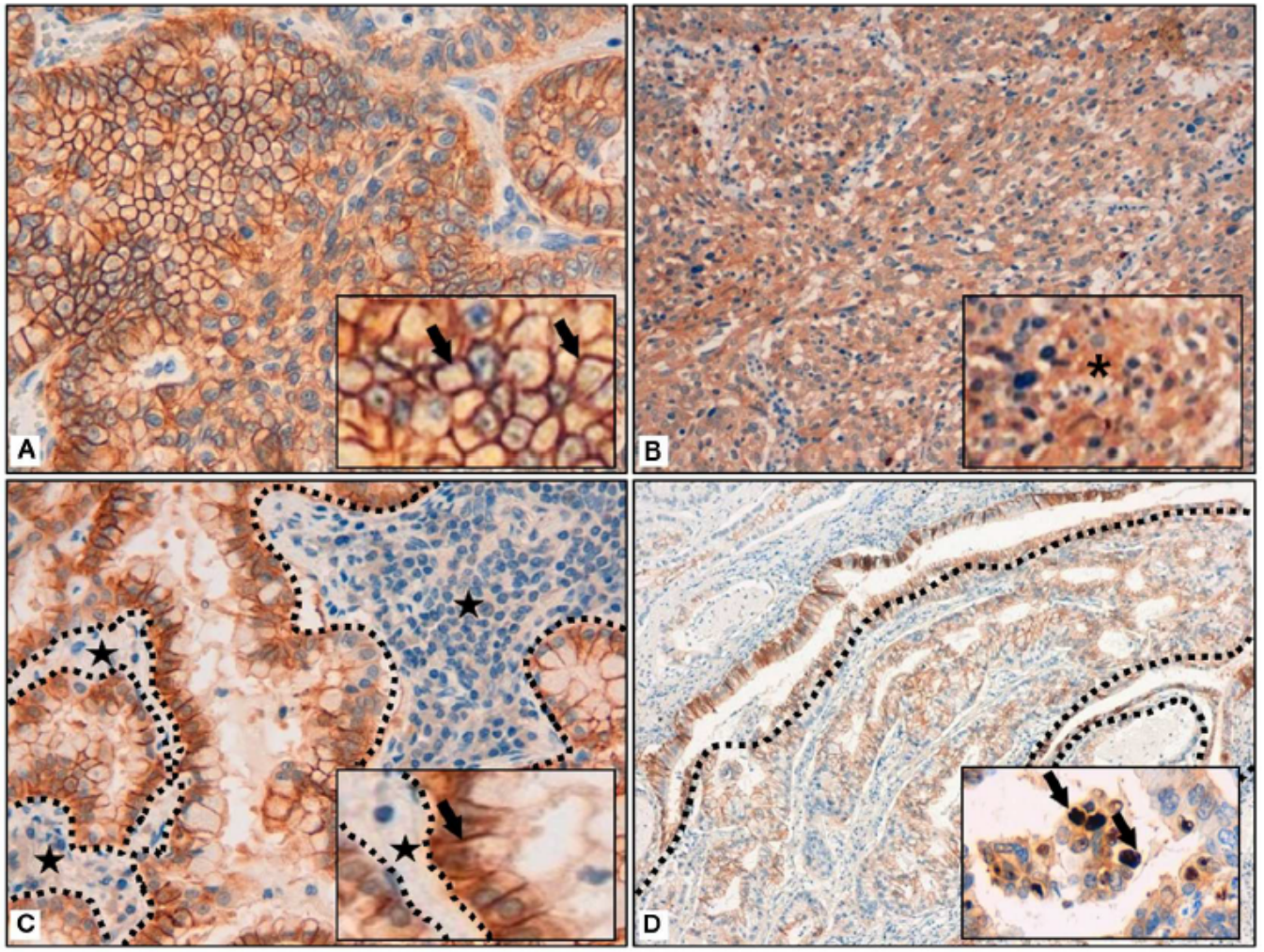
Parameter	IDO2 low		IDO2 high			Total	
	N	%	*N*	%	*p*	*N*	%
	31	16	160	84		191	100

A. Membrane reinforcement

B. Cytoplasmic expression

C. Staining at the tumor-stroma interface

D. Nuclear staining



Mandarano M, et al. Front Immunol. 2020

High expression of IDO2 is associated with a specific histotype

Parameter	IDO2 low		IDO2 high			Total	
	N	%	*N*	%	*p*	*N*	%
	31	**16**	**160**	**84**		**191**	**100**
EXITUS							
Yes	6	9	58	91	0.068	64	34
No	25	20	102	80		127	66
STAGE							
Adc[a] stage						***122***	***64***
I	6	8	68	92	0.964	74	61
II - III	4	8	44	92		48	39
Sqcc[b] stage						***69***	***36***
I	7	26	20	74	0.513	27	39
II - III	14	33	28	67		42	61
HISTOTYPE							
Adc[a]	10	8	112	92	**<0.001**	122	64
Sqcc[b]	21	30	48	70		69	36
Adc[a] pattern						***122***	***64***
Other than solid	6	6	89	94	0.155	95	78
Solid	4	15	23	85		27	22

91% of patients who died from NSCLC present an high IDO2 expression

64% of NSCLC total cases are adenocarcinomas

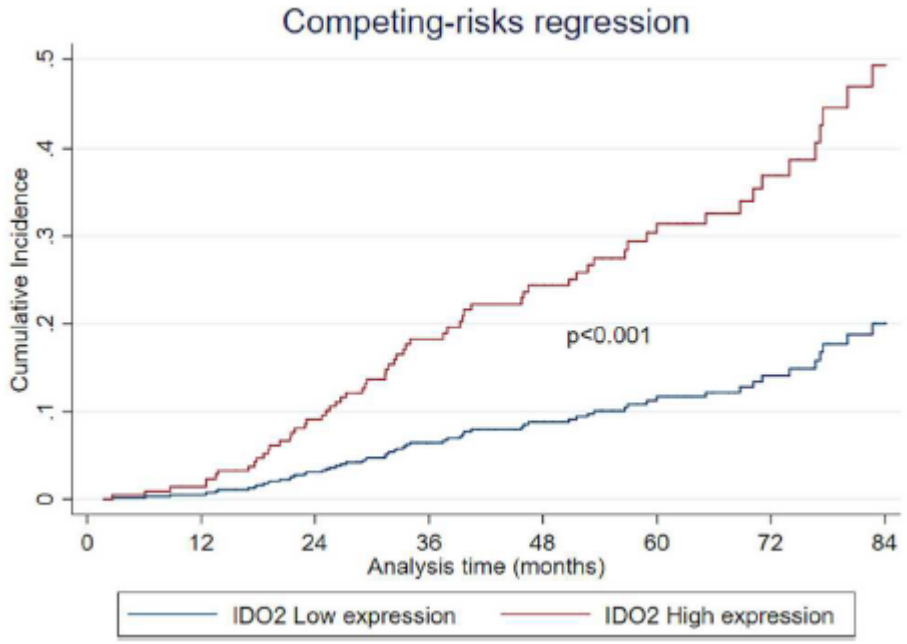
High expression of IDO2 is associated with high PD-L-1 among squamous carcinoma

Parameter	IDO2 low		IDO2 high			Total	
	N	%	*N*	%	*p*	*N*	%
	31	**16**	**160**	**84**		**191**	**100**
PD-L1							
***Adc*[a]**						***122***	***64***
Low	6	6	96	94	**0.035**	102	84
High	4	20	16	80		20	16
***Sqcc*[b]**						***69***	***36***
Low	19	40	29	60	**0.012**	48	70
High	2	10	19	90		21	30

No association between IDO2 expression and TIL density or IDO1 expression

Increased probability of death from NSCLC in patients expressing high levels of IDO2

Parameter	Univariate analysis			Multivariate analysis		
	SHR[a]	*p-value*	*95% CI*[b]	*SHR*[a]	*p-value*	*95% CI*[b]
IDO2						
Low	ref	–	–	ref	–	–
High	2.64	**0.028**	(1.11–6.31)	2.94	**0.011**	(1.28–6.77)



18% in IDO2^{H}

28% in IDO2^{H}

Conclusions

A consistent percentage (84%) of NSCLC has an intense membranous IDO2 staining

High co-expression of both PD-L-1 and IDO2 in the squamous cell carcinomas subgroup

High IDO2 expression correlates with a worse NSCLC outcome

The immunohistochemical assessment of IDO2 together with other molecules (such as PD-L-1) could allow us to **better stratify the risk of patients** with NSCLC, assuming that **more than one biomarker** influences the outcome of these tumors.

Acknowledgments

Section of Pharmacology

Claudia Volpi
Maria Laura Belladonna

Section of Anatomic Pathology and Histology

Angelo Sidoni
Martina Mandarano
Guido Bellezza

Section of Public Health

Umbria Cancer Registry

Department of Thoracic Surgery