Lung cancer and other malignancies -PD-L1 assay, QuIP EQA

Korinna Jöhrens
Institute for pathology
Carl Gustav Cars Universitätsklinikum Dresden, Germany
Medical consultant QuIP

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NSCLC

Harmonization study 1

- 9 German institutes for pathology
- NSCLC slides from tumor-resections
- stainings in reference laboratories DAKO/Ventana
- evaluation according to the Cologne Scores

Cologne Score

A	Assay, Antikörper	Zelltyp	Negativ	Low/Weak	Medium	High/Strong
Nivolumab (α-PD1; BMS)	Dako 28-8	Tumor	0-1%	1-5%	5-10%	≥10%
Pembrolizumab (α-PD1; MSD)	Dako 22C3	Tumor	0-1%	1-50%		≥50%
Atezolizumab (α-PD-L1; Roche)	Ventana SP142	Tumor	0-1%	1-5%	5 - 50%	≥50%
		Immun	0-1%	1-5%	5-10%	≥10%
Durvalumab (α-PD-L1; AstraZeneca)	Ventana SP263	Tumor	1-25%			≥25%
Avelumab (α-PD-L1; Pfizer + Merck)	Dako ...	Tumor	0-1%	?		

B		Negativ	Positiv				
Proportion-Score ('Cologne Score')	Kategorie:	**0**	**1**	**2**	**3**	**4**	**5**
	Cut-Off:	**< 1%**	**≥ 1%**	**≥ 5%**	**≥ 10%**	**≥ 25%**	**≥ 50%**
	Intervall:	0 - 1%	≥1% <5%	≥5% <10%	≥10% <25%	≥25% <50%	≥50% <75%

Scheel et.al: Mod Pathol. 2016 Oct;29(10):1165-72

Results I

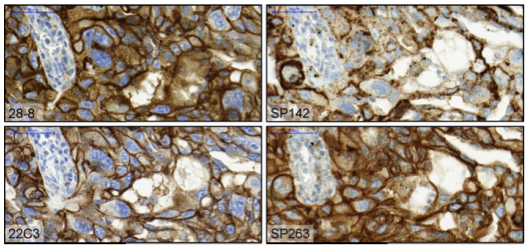
- Good interobserver concordance for tumor cells (light's kappa 0.6-0.8)
- poor interobserver concordance for immune cells

Results II

- membranous staining: complete/incomplete
- cytoplasmatic staining is not counted
- different assays do not show identical staining patterns
- different antibodies stained on the same platform showed similar results

Results III

- 28-8, 22C3, SP263 showed similar patterns
- SP142 showed different pattern: linear, sometimes granular
- 28-8 und 22C3 showed similar proportions
- SP263 stained higher numbers of tumor cells
- SP142 stained lower numbers of tumor cells



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Harmonization study 2

#	Participant	Benchmark	Dako		Ventana		In house
			22C3	28-8	SP142	SP263	
01	Cologne Büttner/Scheel	Ventana Ultra & Dako Autostainer Link 48	x	x	x		X (22C3)
02	Cassel (PNH/Targos) Rüschoff/Jasani	Dako Autostainer Link 48 Ventana Ultra & XT	x	x	x		X (28-8)
03	Göttingen Schildhaus	Dako Autostainer Link 48	x				X (28-8)
04	Berlin Dietel/Lühnens	Ventana Ultra & XT Dako Autostainer Link 48		x		x	X (22C3) (E1L3N)
05	Munic Kirchner/Reu	Ventana Ultra & XT				x	X (28-8)
06	Munic Weichert	Ventana Ultra, Dako Autostainer Plus				x	X (28-8) (22C3)
07	Hamburg Tiemann/Heukamp	Ventana Ultra			x		X (22C3)
08	Heidelberg Schirmacher/ Lasitschka	Ventana Ultra			x		X (SP263?)
XX	Dresden Baretton	Ventana Ultra & XT			x		X (??)

Scheel et al. Histopathology. 2018 Feb;72(3):449-459

IHC: PD-L1	Sites (n)	Readable TMA-cores	Light's kappa (±SD), Tumor proportion score			
			6-step score	3-step score	Proportion cut-off	
					≥ 1%	≥ 50%
22C3, Kit	3	90% (57/63)	0.69 (±0.09)	0.83 (±0.09)	0.87 (±0.01)	0.89 (±0.11)
28-8, Kit	3	94% (59/63)	0.66 (±0.17)	0.80 (±0.15)	0.89 (±0.11)	0.82 (±0.13)
SP263, Kit	4	81% (68/84)	0.66 (±0.22)	0.89 (±0.18)	0.76 (±0.41)[#]	1.00 (±0.00)
SP142, Kit	6	90% (114/126)	0.63 (±0.16)	0.73 (±0.11)	0.71 (±0.16)	0.95 (±0.10)
LDAs	11	82% (189/231)	0.43 (±0.15)	0.50 (±0.18)	0.42 (±0.24)	0.78 (±0.18)

- stainings are reproducible
- similar results in different institutions
- LDT stainings are similar when carefully validated

Scheel et al. Histopathology. 2018 Feb;72(3):449-459

ring trial PD-L1 NSCLC: case selection criteria

Case selection :

- equal distribution of the cases in the following 3 different categories:
- category 1: < 1%: 3 cases
- category 2: ≥ 1% < 49%: 4 cases
- category 3: ≥ 50%: 3 cases

- tumor tissue type: resections

ring trial PD-L1 NSCLC: structure

Internal ring trial:

1. part: lead-institutes (3)
2. part: panel institutes (4)

Open ring trial :

10 cases
2 slides per cases
= 20 slides for the participants

assessment criteria

- 2 points were given for each case
- poor staining results or poor intensity interpretation: 2 points deduction
- technical problems 1 point detraction
- Passed the test: ≥ 18 points

2. Ring trial PD-L1 NSCLC: chosen methods

- free choice of methods
- lead- and panel institutes
  - Dako, Leica, Ventana
  - SP263, SP142, 28-8, 22C3, E1L3N

RT1/2016 and RT2/2017:PD-L1-NSCLC

Participants:

1. 83
2. 94

with success:

1. 60
2. 72

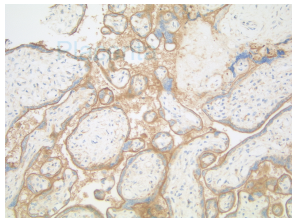
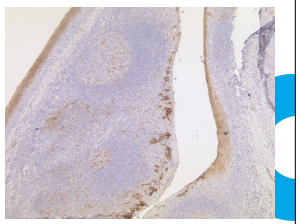
failed:

1. 23
2. 22
1. 72%
2. 77%

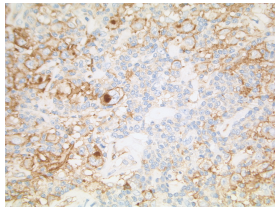
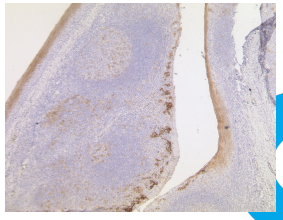
Antibodies used by participants RT1 and RT2

AK	RT1	RT2
E1L3N	22/25 88%	16/21 76%
28-8	13/20 65%	6/11 55%
22C3	9/13 69%	14/21 67%
QR1	4/6	5/11 46%
SP263	2/5	3/4
CAL10	3/4	10/13 77%
ZR3	2/3	5/4
SP142	2/2	0

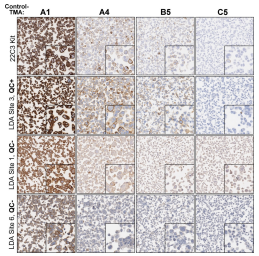
Positive controls

positive controls

LDT calibration with cell-lines



Ring trial PD-L1 NSCLC: interpretation of results

- Free choice of methods
- learning success: 11/15 of the participants, who failed the first ring trial, passed the second (73,3%).

Der Pathologe, in Vorbereitung

Immune therapy with checkpoint-inhibitors
- overview -

	Melanoma		NSCLC		RCC	UC		SCCHN	cHL	MCC	MSI	GC/GEJ
	1st	2nd	1st	2nd	2nd	1st	2nd	2nd	>3rd	2nd	>1st	>2nd
Ipilimumab (anti-CTLA-4)	+	+										
Nivolumab (anti-PD-1)	+[1]	+[1]		+	+		+	+	+[6]		+ (CRC)	
Pembrolizumab (anti-PD-1)	+	+	+[3,7]	+[2]		+[5]	+	+	+[4,6]		+	+[8]
Atezolizumab (anti-PD-L1)				+		+[5]	+					
Durvalumab (anti-PD-L1)			+				+					
Avelumab (anti-PD-L1)							+			+		

[1] = also in combination with Ipilimumab
[2] = only for PD-L1 positive tumors (TPS ≥ 1%)
[3] = only for PD-L1 positive tumors (TPS ≥ 50%)
[4] = FDA: 4. line or refractory (also pediatric pat.)
[5] = if Cisplatin is inapplicable
[6] = EMA: after BV-therapy+ auto-SZT; Pembro additionally after BV-therapy, if auto-SCT is not possible
[7] = in combination with Pemetrexed und Carboplatin in non-squamous NSCLC
[8] = only for PD-L1 positive tumors (CPS ≥ 1)

Until now only FDA

PD-L1-expression is variable between different entities

Entities	PD-L1 expression
malignant melanoma	40-100
NSCLC	35-95
naso-pharynx carcinoma	68-100
glioblastoma, glioma	100
colo-rectal-carcinoma (Adeno)	53
hepatocellular carcinoma	45-93
urothelial carcinoma	28-100
multiple myeloma	93
Ovarian carcinoma	33-80
gastric carcinoma	42
esophageal carcinoma	42
pancreatic carcinoma	39
renal cell carcinoma	15-24
lymphoma	17-94
leukemia	11-42

BMS

PD-L1 expression may vary depending on the previous therapies[1–12]

Agent	Cell Type	Effect on PD-L1 Expression
Radiation therapy[1-3]	Colorectal, breast, melanoma[1]	Up-regulated*†
Cisplatin	Hepatoma[4] HNSCC[5]	Up-regulated*†‡
Paclitaxel	Breast[6], Colorectal, hepatocellular carcinoma[7]	Up-regulated*†
Etoposide[8]	Breast	Up-regulated*
Oxaliplatin[8]	Plasmacytoid dendritic cells	Up-regulated*
Doxorubicin[9]	Breast	Down-regulated*†‡
Gefitinib	NSCLC	Down-regulated*†‡[10] Up-regulated§[11]
Sunitinib / Pazopanib[12]	Metastatic RCC	Down-regulated§

* PD-L1 expression determined by flow cytometry. † PD-L1 expression determined by qRT-PCR or transcriptomeic profiling.
‡ PD-L1 expression determined by western blots. § PD-L1 expression determined by IHC. ¶In tumors resistant to radiation + anti-CTLA-4.

HNSCC = head and neck squamous cell carcinoma; IHC = immunohistochemistry; NSCLC = non-small cell lung cancer; PD-L1 = programmed death ligand 1; RCC = renal cell carcinoma.

1. Dovedi SJ et al. Cancer Res. 2014; 74(19): 5458-5468 2. Deng L et al. J Clin Invest. 2014; 124(2): 687-695
3. Twyman-Saint Victor C et al. Nature. 2015; 520(7547): 373-377 4. Qin X et al. Cell Mol Biol. 2010; 56 Suppl: OL1366-72 5. Qiao J et al. Poster presentation at AACR 2014. 3750 6. Zhang P et al. Mol Immunol. 2008; 45(5):1470-1476 7. Gong W et al. J Immunother. 2011; 23(5): 295-299 8. Tel J et al. Cancer Immunol Immunother. 2012; 61(7): 1101-1111 9. Ghebeh H et al. Breast Cancer Res. 2010; 12(4): R48 10. Lin K et al. Biochem Biophys Res Commun. 2015; 463(1-2): 95-101. 11. Omori S et al. Abstract presented at ASCO 2015 Annual Meeting. e22118 12. Sharpe K et al. Clin Cancer Res. 2012; 18(24): 6924-6934

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PD-L1-Testing

ASSAY						
Characteristica	Nivolumab	Nivolumab	Pembrolizumab	Pembrolizumab	Atezolizumab	Durvalumab
company	Bristol-Myers Squibb		MSD		Roche	AstraZeneca
Target of the therapeutic Antibody	PD-1 (extracellular domain)	PD-1 (extracellular domain)	PD-1 (extracellular domain)	PD-1 (extracellular domain)	PD-L1 (intracellular domain)	PD-L1 PD-1 (extracellular domain)
PD-L1-POSITIVITY						
IHC-Assay producer	Dako	Ventana	Dako	Ventana	Ventana	Ventana
IHC-antibody-clone	28-8 (rabbit)	SP263 (rabbit)	22C3 (mouse)	SP263 (rabbit)	SP142 (rabbit)	SP263 (rabbit)
Evaluation PD-L1-expression	Tumor cells (TCs)	Tumor cells (TCs)	Tumor cells (TCs) and stroma	Tumor cells (TCs) and stroma	Tumor-infiltrating immune cells (TICs) & Tumor cells (TCs)	Tumor cells (TCs)
„Cut-off" inNSCLC	≥ 1%, ≥ 5%, ≥ 10% TC	≥ 1%, ≥ 5%, ≥ 10% TC	≥ 1%, ≥ 50% TC (or every tumor-stroma-cell)	≥ 1%, ≥ 50% TC (or every tumor-stroma-cell)	IHC 2+ ≥ 5 % to ≤ 10 % TC or TIC or IHC 3+ ≥ 10% TC or TIC	≥ 25% TC

Depending on the therapeutic antibodies different PD-L1-Assays with different clones and specific evaluation algorithm regarding IHC-stainings are developed.

Modified from Aaron R. Hansen & Lillian L. Siu, PD-L1 Testing in Cancer, Challenges in Companion Diagnostic Development: JAMA Oncology January 2016 Volume 2, Number 1

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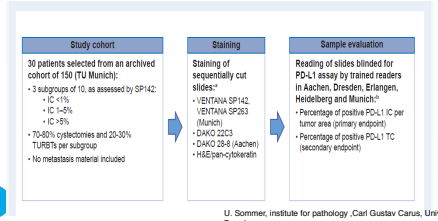
Harmonization study urothelial carcinoma

Design – PLACU-Study

Analytical comparison of the percentage of PD-L1 stained immune cells (per tumor area) and PD-L1 stained tumor cells in 30 patients with advanced urothelial carcinoma.
For comparison, the clinical trials validated assays Ventana SP142, Ventana SP263, Dako 28-8 and Dako 22C3 were used. All 5 evaluators were trained on the evaluation of immune cells (eminating on the assay SP142).

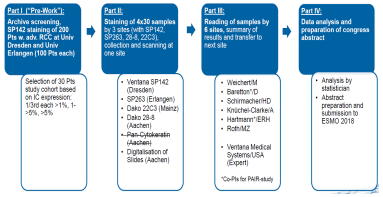


U. Sommer, institute for pathology ,Carl Gustav Carus, Universitätsklinikum Dresden

Harmonization study renal cell carcinoma

Roche PAIR-Studie

Analytical comparison of the percentage of PD-L1 stained immune cells (per tumor area) and PD-L1 stained tumor cells in 30 patients with advanced clear cell renal cell carcinoma.
Staining with the clinically validated PD-L1 assays Ventana SP142 and SP263, Dako 28-8 and 22C3 (each according to manufacturer's protocols).



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Harmonization study urothelial-, gastric- HNSCC-carcinoma

MSD
6 German institutes for pathology
In progress

Questions

A positive PD-L1 staining is localized in/at the

A) nucleus
B) cytoplasm
C) membrane

2. SP142 stained

A) a lower number of tumor cells
B) a higher number of tumor cells
C) similar to SP263

3. Which tissue should be used as a positive control for PD-L1:

A) placenta
B) tonsil
C) classical Hodgkin lymphoma