A PD-L1 IHC 28-8 PharmDx ring trial on metastatic melanoma: practical aspects

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Conflicts of interest

This ring trial was funded by Bristol-Myers Squibb Belgium

Background & objectives

- Evaluation of PD-L1 IHC staining is challenging
- A Belgian ring trial for PD-L1 IHC staining in melanoma was organized by the pathology department of Antwerp University Hospital
- Aim:
  - evaluation of reproducibility of PD-L1
  - give feedback in order to standardize the interpretation of PD-L1 staining protocols for melanoma testing

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Melanoma PD-L1 ring trial (RT)

Organized between Dec 2017 – Jul 2018

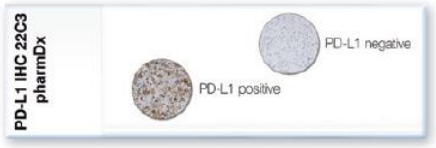
Contained 6 samples (metastasized melanoma)

- 3 cases with <5% PD-L1
- 3 cases with ≥5% PD-L1

Participation of 14 different Belgian laboratories (1 lab participated with 2 methods)

Melanoma PD-L1 ringtrial - Set-up

- Set-up:
  1. First and last slide of all samples were stained with the **reference method** (PD-L1 28-8 pharmDx protocol on an Autostainer Link 48.
     - Inclusion of control cell line to confirm technical performance of the run



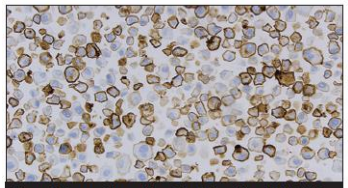


Figure 7: Acceptable staining of positive pellet of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20x magnification).

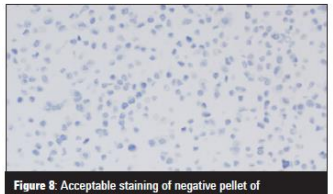
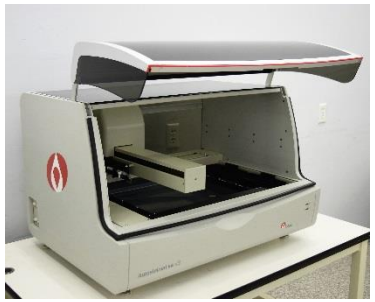


Figure 8: Acceptable staining of negative pellet of PD-L1 IHC 22C3 pharmDx Control Cell Line Slide (20x magnification).



     - Inclusion of blanc control for each sample

  2. Blank slides were sent to participating sites for staining. **PD-L1 stained slides + interpretation of pathologist** were sent back for evaluation.

  3. Stained slides were compared with slides stained with reference method.

     Evaluations of participating site was compared with evaluation of 2 (certified) reference pathologists.

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Melanoma PD-L1 ringtrial – Set-up

Criteria for evaluation of the slides / Scoring system

- Tumor percentage score (TPS)

- Criteria for interpretation of the PD-L1 staining: manual of the PD-L1 IHC 28-8 pharmDx assay

- Cut-off:

  <5%

  ≥5%

- Average range:

  <1%, 1–5%, 5–15%, 15–30%, 30–50%, and ≥50%

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Melanoma PD-L1 ringtrial – Technical part

RESULTS

	Sample	PD-L1 expression with PD-L1 IHC 28-8 pharmDx assay (%)	Average range*	Sites** with good staining***, n (%) (n = 15)	Sites** with good staining****, n (%) (n = 15)	Common mistakes (FP/FN)***	Remark
Low	18S71	<1%	<1% (cutoff: <5%)	4 (27%)	15 (100%)	11 FP / 0 FN (1–5%, n = 11)	11 FP because of melanin
	18S72	<1%	<1% (cutoff: <5%)	9 (60%)	15 (100%)	6 FP / 0 FN (1–5%, n = 6)	
Moderate	18S74	5%	5–15% (cutoff: ≥5%)	10 (67%)	10 (67%)	0 FP / 5 FN (1–5%, n = 5)	Low intensity of staining
	18S93	4%	1–5% (cutoff: <5%)	14 (93%)	15 (100%)	0 FP / 1 FN (<1%, n = 1)	Low intensity of staining
High	18S73	10%	5–15% (cutoff: ≥5%)	6 (40%)	15 (100%)	9 FP / 0 FN (15–30%, n = 9)	9 FP because of melanin
	18S76	20%	15–30% (cutoff: ≥5%)	0 (0%)	8 (53%)	0 FP / 15 FN (<1%, n = 3; 1–5%, n = 4; 5–15%, n = 8)	Educational sample

FN, false negative; FP, false positive; PD-L1, programmed death ligand 1.
*Based on CheckMate 067.
**One site participated using two protocols and is counted as two sites for the purposes of this analysis.
**Based on average range.
****Based on cutoff.

CONCLUSION: Overall, the staining of most sites is within the correct cutoff

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Melanoma PD-L1 ringtrial – Practical part

RESULTS

Sample	Sites* with discrepant** scoring, % (n = 15)
18S71	33% (5 FP, 0 FN)
18S72	13% (2 FP, 0 FN)
18S74	47% (3 FP, 4 FN)
18S93	27% (4 FP, 0 FN)
18S73	20% (0 FP, 3 FN)
18S76	40% (2 FP, 4 FN)

FN, false negative; FP, false positive.
*One site participated using two protocols and is counted as two sites for the purposes of this analysis.
**Discrepant refers to the assigned score with respect to the 5% cutoff.

CONCLUSION: - melanin causes an over-estimation
- cases close to the 5% cut-off: difficult interpretation
- average disconcordance: 34,5%

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Melanoma PD-L1 ringtrial – AB and platforms

RESULTS

Site*	Score based on average range	Score based on cutoff	Antibody clone	Platform	Protocol	Detection kit
1	1 FP / 1 FN	1 FN	28-8	Omnis	In house	DAB
2	1 FN	1 FN	22C3	BenchMark ULTRA	In house	DAB
3	3 FP / 1 FN	1 FN	22C3	Omnis	In house	DAB
4	1 FP	–	22C3	BenchMark ULTRA	In house	DAB
5	2 FP	–	SP263	BenchMark ULTRA	Kit	DAB
6	1 FN	1 FN	22C3	Autostainer	Kit	DAB
7	3 FP	–	SP263	Autostainer	In house	ALP
8	1 FP / 1 FN	–	22C3	Omnis	In house	DAB
9	3 FP	–	22C3	BenchMark ULTRA	In house	DAB
10	2 FP	–	22C3	BenchMark ULTRA	In house	DAB
11	1 FP	–	22C3	BenchMark ULTRA	In house	DAB
12	2 FP	–	22C3	BenchMark ULTRA	In house	DAB
13	3 FP	–	22C3	BenchMark ULTRA	In house	ALP
14	2 FP / 1 FN	1 FN	22C3	Omnis	In house	DAB
15	2 FP	–	22C3	Benchmark ULTRA	In house	DAB

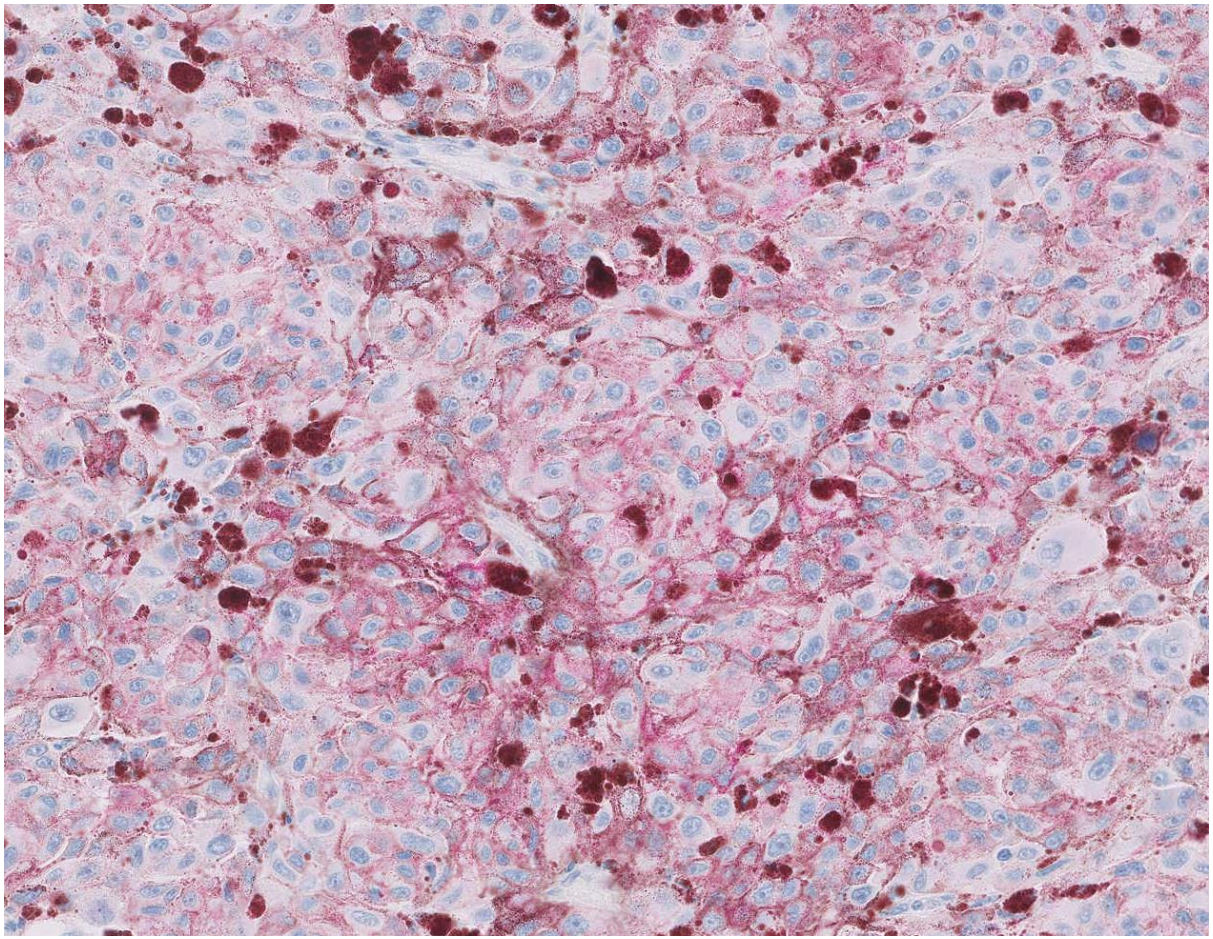
ALP, alkaline phosphatase; DAB, 3,3'-diaminobenzidine; FN, false negative; FP, false positive.
*One site participated using two protocols and is counted as two sites for the purposes of this analysis.

Melanoma PD-L1 ringtrial – AB and platforms

CONCLUSION:

- 80% used the 22C3
- Benchmark most popular platform with 60%
- 92% of the laboratories used an in-house protocol
- Only 2 laboratories used ALP as detection kit
- Overestimation again due to intense hyperpigmentation

PD-L1 28-8 IHC: detection kit ALP



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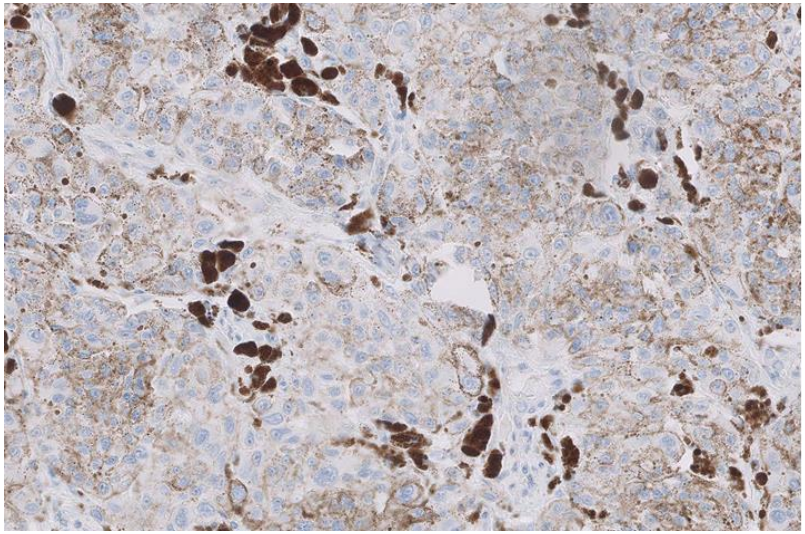
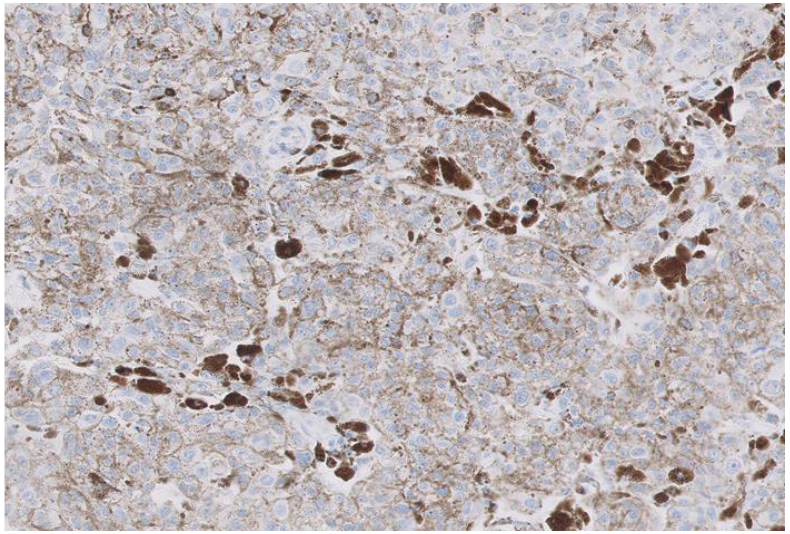
PD-L1 28-8 IHC: negative control and staining





Melanoma PD-L1 ringtrial – General remarks

- PD-L1 IHC staining resulted in similar conclusions in about 65% of cases, independent of the platforms and clones used

- Abundant melanin deposition causes overestimation
  → use ALP or magenta as detection kit
  → use negative control slide
- Histiocytic reaction
  → use HE staining

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Melanoma PD-L1 ringtrial – General remarks

- Most challenging cases are around 5% cut-off
  → evaluate the whole slide and not only the hot spots
  → score each field separately
  → ask for a second opinion from another experienced pathologist

THANK YOU FOR YOUR ATTENTION

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We thank the Belgian laboratories and pathologists who participated in this ring trial

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