PD-L1 expression testing in Urothelial Carcinoma

Dr Philippe Taniere
Molecular Pathology Diagnostic Services
University Hospitals Birmingham

NICE recommendations

- Atezolizumab is recommended for use within the Cancer Drugs Fund as an option for untreated locally advanced or metastatic urothelial carcinoma in adults when cisplatin-containing chemotherapy is unsuitable,
  - only if: their tumours express PD-L1 at a level of 5% or more
- Pembrolizumab is recommended for use within the Cancer Drugs Fund as an option for untreated locally advanced or metastatic urothelial carcinoma in adults when cisplatin-containing chemotherapy is unsuitable,
  - only if: their tumours express PD-L1 with a combined positive score of 10 or more

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Disclosures

- **I have received fees for talks or attending advisory board meetings from:**
- Agilent, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, MSD, Merck Serono, Novartis, Pfizer, Roche and Ventana

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NICE recommendations

- Atezolizumab is recommended as an option for treating locally advanced or metastatic urothelial carcinoma in adults who have had platinum-containing chemotherapy.
  - only if: atezolizumab is stopped at 2 years of uninterrupted treatment or earlier if the disease progresses and
- Pembrolizumab is recommended for use within the Cancer Drugs Fund as an option for treating locally advanced or metastatic urothelial carcinoma in adults who have had platinum-containing chemotherapy.
  - only if: pembrolizumab is stopped at 2 years of uninterrupted treatment or earlier in the event of disease progression

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PD-L1 testing in routine practice at UHB

- PD-L1 IHC 22C3 pharmDx (Agilent) on Autostainer Link 48
- PD-L1 IHC 28-8 pharmDx (Agilent) on Autostainer Link 48
- Ventana *PD-L1 (SP263)* Assay on Benchmark Ultra
- Ventana PD-L1 (SP142) Assay on Benchmark Ultra

PD-L1 interpretation training completed

- Lung 22C3 pharmDx
- Lung Ventana *PD-L1 (SP263)* Assay
- Lung Ventana PD-L1 (SP142) Assay
- Melanoma 28-8pharmDx
- Urothelial Carcinoma Ventana PD-L1 (SP142) Assay
- Urothelial Carcinoma 22C3 pharmDx
- Head and Neck 28-8pharmDx
- Breast carcinoma Ventana PD-L1 (SP142) Assay

PD-L1 testing in routine practice at UHB

Autostainer Link 48

https://www.agilent.com/

Benchmark Ultra

https://usdiagnostics.roche.com

PD-L1 testing in routine practice at UHB

- Lung cancer, 22C3 pharmDx : April 2016-June 2019: 26 000 cases
- Melanoma, 28-8 pharmDx : April 2016-June 2019: 600 cases
- Head and Neck, 28-8 pharmDx : 70 cases
- Urothelial carcinoma: 22C3 pharmDx and Ventana PD-L1 (SP142) Assay : June 2018-June 2019: 744 cases
- Breast carcinoma: Ventana PD-L1 (SP142) Assay: April 2019-June 2019: 90 cases

PD-L1 interpretation is specific to the assay and to the tumour type

- Lung 22C3 pharmDx , 28.8. Ventana *PD-L1 (SP263)* Assay : membranous staining in tumour cells
- Lung Ventana PD-L1 (SP142) Assay : ≥ 50% TC or ≥ 10% IC
- Bladder Ventana PD-L1 (SP142) Assay : ≥ 5% IC
- Urothelial Carcinoma 22C3 pharmDx : Combined Positive Score (CPS)
- Melanoma 28-8 pharmDx : membranous staining in tumour cells (5%)
- Head and Neck 28-8 pharmDx : membranous staining in tumour cells (1%)

PD-L1 testing in Urothelial Carcinoma

Pitfalls for PD-L1 assessment

- Spatial heterogeneity
  - Adequacy of biopsies/cytology?
  - Primary or metastasis? (or both?)
- Temporal heterogeneity
  - Impact of chemotherapy
  - Impact of immunotherapy
  - Impact of immune status

2 drugs-2 PD-L1 tests

Assessment with Ventana PD-L1 SP142 assay

- The proportion of tumour-infiltrating immune cells (IC) covering the tumour areas is assessed.
- Tumour is positive for PDL1 expression and patient is suitable for Atezolizumab therapy when there is discernible PD-L1 staining of any intensity in tumour-infiltrating immune cells (lymphocytes, macrophages and granulocytes) covering at least or more of 5% of tumour area.

Ventana PD-L1 SP142 assay for urothelial carcinoma

Table 3: Tumor-Infiltrating Immune Cell (IC) Characteristics	
Type of cells showing staining	Lymphocytes, macrophages, dendritic cells, and granulocytes
Type of cells included in scoring	Lymphocytes, macrophages, dendritic cells, and granulocytes
Pattern	Aggregates in stroma, single cells dispersed among tumor cells with punctate, linear or circumferential staining
Denominator for scoring	Tumor area

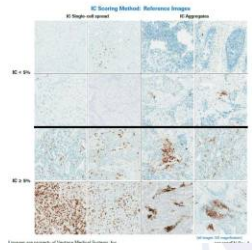
VENTANA PD-L1 (SP142) Assay
Interpretation Guide for Urothelial Carcinoma

Ventana PD-L1 SP142 assay for urothelial carcinoma

Table 1: VENTANA PD-L1 (SP142) Assay Scoring Algorithm for Urothelial Carcinoma	
Tumor-Infiltrating Immune Cell (IC) Staining	**PD-L1 Expression**
Absence of any discernible PD-L1 staining (OR) Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering < 5% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	< 5%
Presence of discernible PD-L1 staining of any intensity in tumor-infiltrating immune cells covering ≥ 5% of tumor area occupied by tumor cells, associated intratumoral, and contiguous peritumoral stroma	≥ 5%

VENTANA PD-L1 (SP142) Assay
Interpretation Guide for Urothelial Carcinoma

Ventana PD-L1 SP142 assay for urothelial carcinoma



VENTANA PD-L1 (SP142) Assay
Interpretation Guide for Urothelial Carcinoma

Assessment with 22C3 PharmDx

- PDL1 expression is determined by Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumour cells, lymphocytes and macrophages) divided by the total number of viable tumour cells, multiplied by 100.
- Tumour is positive and patient is suitable for Pembrolizumab therapy when CPS is at or above 10 (Maximum score is 100)

22C3PharmDx Urothelial Carcinoma

Table 2: CPS Denominator Inclusion/Exclusion Criteria

Tissue Elements	Included in the Denominator	Excluded from the Denominator
Tumor Cells	All viable tumor cells including: • High grade papillary carcinoma • Carcinoma in situ (CIS) • Any lamina propria, muscularis, or serosal invasion • Metastatic carcinoma	• Any necrotic or non-viable tumor cells • Low grade papillary carcinoma[†1]
Immune Cells	Not included	All immune cells of any type
Other Cells	Not included	• Normal cells • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris

†1 If the tumor consists entirely of low grade papillary carcinoma, the result should be flagged as such

22C3PharmDx Urothelial Carcinoma

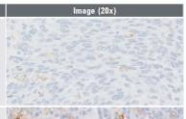
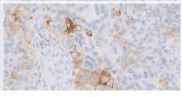
Table 1: CPS Numerator Inclusion/Exclusion Criteria

Tissue Elements	Included in the Numerator	Excluded from the Numerator
Tumor Cells	Convincing partial or complete linear membrane staining (at any intensity) of viable urothelial carcinoma tumor cells including: • High grade papillary carcinoma • Carcinoma in situ (CIS) • Any lamina propria, muscularis, or serosal invasion • Metastatic carcinoma	• Non-staining tumor cells • Tumor cells with only cytoplasmic staining • Low grade papillary carcinoma[†]
Immune Cells	Membrane and/or cytoplasmic* staining (at any intensity) of mononuclear inflammatory cells (MICs) within tumor nests and adjacent supporting stroma[‡]: • Lymphocytes (including lymphocyte aggregates) • Macrophages[‡] Only MICs directly associated with the response to the tumor are scored.	• Non-staining MICs • MICs (including lymphoid aggregates) associated with ulcers, chronic cystitis, and other processes not associated with the tumor • MICs associated with normal structures • Neutrophils, eosinophils, and plasma cells • BCG*-induced granulomas
Other Cells	Not included	• Normal cells • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris

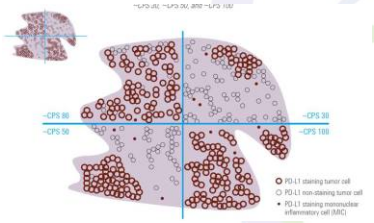
* In **MICs**, membrane and cytoplasmic staining are often indistinguishable due to high nuclear to cytoplasmic ratio. Therefore, membrane and/or cytoplasmic staining of MICs are included in the score. † **Adjacent MICs** are defined as being within the same 20x field as the tumor. However, MICs that are NOT directly associated with the response to the tumor should be excluded. ‡ Macrophages and histiocytes are considered the same cells. § If the tumor consists entirely of low grade papillary carcinoma, the result should be flagged as such. ¶ BCG = Bacillus Calmette-Guérin

22C3PharmDx Urothelial Carcinoma

Table 3: CPS and PD-L1 Expression

CPS	PD-L1 Expression	Image (20x)
< 10	CPS is less than 10	
≥ 10	CPS is greater than or equal to 10	

22C3PharmDx Urothelial Carcinoma



22C3PharmDx Urothelial Carcinoma

UHB experience

- Between 26.07.2018 and 21.06.2019
  - 744 cases referred
  - 21 cases not suitable (3%)
  - 552 cases for Pembrolizumab
  - 440 cases for Atezolizumab
  - 277 cases for both
- turn around time: 3 to 6 working days

22C3PharmDx Urothelial Carcinoma

Table 4: PD-L1 Prevalence in Patients with Urothelial Carcinoma Enrolled in KEYNOTE-052

PD-L1 Expression	CPS < 10	CPS ≥ 10
Prevalence (n)*	69.5% (251)	30.5% (110)

* 9 patients had unknown PD-L1 status

Table 5: PD-L1 Prevalence in Previously Treated Patients with Urothelial Carcinoma Enrolled in KEYNOTE-045

PD-L1 Expression	CPS < 10	CPS ≥ 10
Prevalence (n)†	66.8% (362)	30.2% (164)

† 16 patients (10 from pembrolizumab and 6 from chemotherapy) had unknown PD-L1 status

22C3PharmDx Urothelial Carcinoma

UHB experience

- Ventana PD-L1 (SP142) Assay :
  - 36% positive (more than 5%)
- 22C3 PharmDx assay:
  - 28% positive (CPS >10)

UHB experience

- Correlation between both assays
  - 240/277 cases with concordant results (87%)
  - 36/277 cases with discrepant results (13%)
    - 7 cases Pos for Pembrolizumab and Neg for Atezolizumab
    - 29 cases Neg for Pembrolizumab and Pos for Atezolizumab

Perspective: more predictive markers?

- MisMatch Repair deficiency (MSI-H/loss of loss of MMR protein expression): agnostic predictive marker for Pembrolizumab (FDA approved): not licensed in England
- Tumour Mutation Burden (TMB)
  - Not a clinically validated marker in urothelial carcinomas

Perspective

- Durvalumab (Imfinzi) possibly getting licensed soon?
  - PD-L1 assessment: SP263
    - IC >25% and TC >25%