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PD-L1 expression as predictor of immunotherapy eligibility in penile squamous cell carcinoma patients

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We use this protocol and it's working

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Abstract

We investigated the correlation between PD-L1 expression and poor prognosis. We examined the correlation between PD-L1 expression and survival of penile SqCC and analyzed the adjusted hazard ratios (HR) of the patients with PD-L1 expression compared to anatomical stage of the tumor.

Materials

-MD21R clone (Medaysis CA)
-Rabbit PD-L1 antibody
-DAB mixture
-Hematoxylin

PD-L1 Antibody Preparation

1 Reagent used to stain PD-L1 in this IHC staining was MD21R clone (Medaysis CA), ready to use (rabbit PD-L1 antibody)

2 PD-L1 antibody is diluted with ratio 1 uL PD-L1: 100 uL IHC diluent and then mixed for 5 minutes. 5m

Pretreatment

3 Pretreatment was done with EDTA pH8.0, 15 minutes using a Pressure Cooker 1h 15m

00:15:00 Pressure cooker

OR

01:00:00 30 – 60 minutes using a water bath 95 °C to 99 °C

4 Deparaffination and rehydration were done by using Bond Dewax Solution for 3 times, alcohol for 3 times, and Bond Wash Solution for 3 times.

5 We retrieved the antigen by using Bond Epitope Retrieval Solution I for 4 times

PD-L1 and Sample Mixing and Detection

6 We mixed the solution with PD-L1 (Medaysis) primary antibody for 1 hour. 1h

7 The detection was done by using post primary procedure for 8 minutes, Bond Wash Solution for two times, and polymer for eight minutes. We used DAB mixture for staining and hematoxylin for counterstaining. 16m

00:08:00 post primary procedure

Bond Wash Solution Two times

00:08:00 polymer

Analysis

8 PD-LI expression was assessed on cytoplasm and/or tumor cell membranes and TILs. The positive control used was the placenta.

9 PD-LI expression is considered positive if it has a score of +2 or +3 and is considered negative if it has a score of +1 and 0. Intensity measurement then given a score of 0-3 (0= none; 1= weak; 2= moderate; and 3= strong).

10 The percentage of stained cells was assessed on a scale of 0-100%.
Percentage of stained tumor cells A= percentage of tumor cells intensity in 3+ {3 x (%Cells 3+)}, B= percentage of tumor cells intensity in 2+ {2 x (%Cells 2+)}, C= percentage of tumor cells intensity in 1+ {1 x (%Cells 1+)}

11 Semi-quantitative assessment refers to research by Chovanec *et al.* who used histoscore (H-score)

H-Score = {1 x (%cells 1+) + 2x (%cells 2+) + 3x (%cells 3+)}

12 Total value obtained ranges from 0 to 300, thus PD-L1 expression is categorized into “1” for negative (0 – 99) and “2” for positive (100 – 300).