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.

Pretreatment

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

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

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.

PD-L1 expression was assessed on cytoplasm and/or tumor cell membranes and TILs. The positive control used was the placenta.

PD-L1 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).

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+)).

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

\[ H\text{-Score} = \{ 1 \times (%\text{cells 1+}) + 2 \times (%\text{cells 2+}) + 3 \times (%\text{cells 3+}) \} \]

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).