2020-07-13 / Letter size

Dear Tae Hyun and Hongming

I want to briefly introduce “Immunoscore” suggested by Galon and colleagues.

I guess you already are familiar with this terminology. In 2018, they published an international validation study using the Immunoscore in Lancet (*Lancet 2018; 391: 2128-2139.)*

|  |  |
| --- | --- |
| Definition of Immunoscore |  |
| EMB00003c541881 | **A) Measurement of Immunoscore using IHC at tumor center and invasive margin13**  Ref-13 Marliot F, Chen X, Kirilovsky A, Sbarrato T, El Sissy C, Batista L et al. Analytical validation of the Immunoscore and its associated prognostic value in patients with colon cancer. J Immunother Cancer 2020; 8. |
| EMB00003c541882 | **B) Clinical significance of Immunoscore compared with clinicopatholgical variables.10**  Ref-10 Pages F, Mlecnik B, Marliot F, Bindea G, Ou FS, Bifulco C et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. *Lancet 2018; 391: 2128-2139.* |
| Other relevant papers on Immunoscore recently published | 11 Pagès F, André T, Taieb J, Vernerey D, Henriques J, Borg C et al. Prognostic and predictive value of the Immunoscore in stage III colon cancer patients treated with oxaliplatin in the prospective IDEA France PRODIGE-GERCOR cohort study. Ann Oncol 2020; 31: 921-929.  12 Sinicrope FA, Shi Q, Hermitte F, Zemla TJ, Mlecnik B, Benson AB et al. Contribution of Immunoscore and Molecular Features to Survival Prediction in Stage III Colon Cancer. JNCI Cancer Spectr 2020; 4: pkaa023. |

However, to estimate Immunoscore, we need to do additional staining using CD3 and CD8. I thought that without IHC staining, it would be great work to make a computational algorithm to mimic the Immunoscore using only H&E slides.

The project would be making a deep learning model to measure Ai\_ImmunoClassifier to act similar function like Immunoscore.

There would be three scanned slides per each person, one is H&E slide, the other would be CD3, CD8. These slides were newly generated ones with adjacent sections. This would be our strength to do this work.

I believe that we need more discussions on this subject, I just explain my simple and unrefined idea to make an algorithm.

1)Is it possible to make tiles just the same size and locations for three scanned slides per patient?

**Reply: Yes. If the three slides are neighboring slides cut from the same tumor tissue. We can make the tiles with the same size and locations, although there exist negligible mismatches between slides.**

2)Could you match one tile per one tile from H&E slide with the other CD3 or CD8 ones?

**Reply: Yes. I think we could do it.**

3)Is it possible to decide one tile from H&E having a certain number of CD3 or CD8 together?

(In measuring the Immunoscore by the original paper, they did not discriminate CD3 and CD8, just measure the densities of each respectively. So I believe that we do not differentiate CD3 and CD8 at this point. This would make our work easier.)

**Reply: Yes. We can do this. But to my understanding, at current stage we can do it at the coarse level. For our TILs detection now, we predict each tile as containing tils or not. Then we can generate a tils detection map. Using CD3 and CD8 slides, we can also divide them into tiles and then count if there exist CD3 OR CD8 in the tiles. After that, we can make another CD3&CD8 map. Finally, we can make comparisons between H&E-based tils map and CD3&CD8 map. I think this is a good now to analyze at coarse level which is computational efficient.**

**In the next step (for long-terms), we need to analyze slides in cellular level. By that I mean we need to build the model to detect cell nuclei and classify them into different categories such as cancer cell nuclei and lymphocytes. Then based on the counts of lymphocytes, we can estimate the immunescore. To do this, we need devote times to develop nuclei detection and classification models.**

4)Then we can make annotated files that include CD3 or CD8 per tile (and we need to decide which level we should make a cut-off) and this might be used as experimental files to generate the convolutional neural network.

**Reply: Yes. We would need decide cut-off values.**

5)In doing 4), we can compare the CD3/CD8 annotation tile with TILs detection results you already generated.

**Reply: Yes. I think it is feasible.**

**I downloaded your shared slides, but it has only one patient slide with H&E, and IHC stained slides. If we want to verify if H&E based TILS detection are consistent with CD4&CD8 slides, I think we need more slides, e.g., 20 or 30 patient slides. For this verification: (1) we can show our tils detection is convincing. (2) using H&E slides, we can estimate immune scores directly.**

**Those are my understandings. IF not correct and enough, please correct me.**

These are my initial suggestion. As you can find **ADDITIONAL CD4 and FOXP3** staining results. I believe that we can make a new AI based algorithms using IHC staining slides together. This will be another project we can consider LATER.

Best

Jeonghyun