University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship.

1. Student

Surname: Eriksson Forename: Hanna

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2. Supervisor:

Surname: Edwards Forename: Joanne

E-mail address: joanne.edwards@glasgow.ac.uk

3. Research Project Report

3.1 Project Title (maximum 20 words): The expression of LAG-3 and TIM-3 in colorectal cancer.

3.2 Project Lay Summary (copied from application):

Colorectal cancer (CRC) is one of the most common types of cancer. It is the third most common cancer in men and the second most common cancer in women. It is increasingly recognized that an immune response is an important factor associated with colorectal cancer. In this project we will investigate the role of immune checkpoints in colorectal cancer and how they might impact patient outcome and survival. To do this we will assess expression of immune checkpoint proteins to see if they are linked with inflammation associated with cancer and clinical outcome measures.

3.3 Start Date: 04/06/2018 Finish Date: 26/07/2018

3.4 Original project aims and objectives (100 words max):

The aim of this project was to investigate possible relationships between the expression of four different immune checkpoint proteins (LAG-3, TIM-3, PDL-1 and PD-1) and survival rates in patients with colorectal cancer. Immunohistochemistry was used to stain for LAG-3 and TIM-3 on tissue microarrays (TMAs) of patients from four different cohorts, which are available for future scoring.

Previously stained TMAs showing PDL-1 and PD-1 expression were scored using the weighted histoscore method and by counting positive lymphocytes. These results were analysed using SPSS, producing Kaplan-Meier plots showing the relationship between PDL-1 and PD-1 expression and disease-specific patient survival rates.

3.5 Methodology: Summarise and include reference to training received in research methods etc. (250 words max):

In the experiment, standard immunohistochemistry procedure was used to stain for expressed LAG-3 and TIM-3 proteins in tissue of patients from different cohorts (AP-CRC-TMA, DM-CRC-TMA, JP-CRC-TMA and GRI-CRC-TMA). This procedure includes techniques such as pipetting, pH-calibration, preparation of solutions and mounting of slides. Trial runs on tissue samples and practice TMAs were conducted to test the antibody concentration used for each protein, to create staining of appropriate strength. Finally, TMAs containing tissue samples from patients were stained and scanned to enable future scoring. Training in experimental techniques was given by Dr Joanne Edwards and Dr Jean Quinn.[1]

TMAs stained for PDL-1 from two cohorts, DM-CRC-TMA[2] (n = 182) and AP-CRC-TMA[3] (n = 758), were scored using the weighted histoscore method (WHS) to assess expression of PDL-1 in tumour cells. Additionally, PDL-1 and PD-1 positive lymphocytes in stroma and tumour of the tissue samples were counted. Training in histology and scoring was given by Dr Joanne Edwards.

The scores were combined with patient data and analysed using SPSS. To define the cut-off points of highest specificity vs sensitivity of the collected scores against disease-specific survival, ROC curves were produced. Kaplan-Meier plots were created for the results generated, to investigate the relationship between expression of proteins in different locations and survival rates of patients.

[1] Additionally, immunohistochemistry was conducted on other unrelated projects, staining for phospho-HSF-1 and NRF-2 proteins in colorectal tissue. Project supervisor: Dr Jean Quinn.
[2] Diagnosed colorectal cancer stages 1-2 (early stages)
[3] Diagnosed colorectal cancer stages 3-4 (later stages)

3.6 Results: Summarise key findings (300 words max). Please include any relevant tables or images as an appendix to this report:

The mean expression of PDL-1 in tumour cytoplasm is higher in early (184.0379 WHS; Table 1) than in the later stages of CRC (121.7969 WHS; Table 4). Mean PDL-1 and PD-1 positive lymphocyte counts are also higher in early (Table 1) than in late stages (Table 4).

In patients with early stages of CRC, no significant difference in survival rate between high and low expression of PDL-1 in tumour cytoplasm was found ($p = 0.100$; Figure 1). Additionally, no significant relationship was found between the number of lymphocytes expressing of PDL-1 or PD-1 (in stroma or tumour) and survival rate (Figure 2-6). However, a statistically significant relationship was found between the combined PD-1 (stroma & tumour) scores in patients in early stages and survival ($p = 0.007$; Figure 7). Pairwise comparison of statistics for PD-1 scores at different levels of expression show that the highest statistical significance is between the low and the medium group (Table 3).

In later stages of CRC, high levels of PDL-1 in tumour cytoplasm showed a relationship with higher survival rates (approx. 70% after 200 months). Low levels of PDL-1 correlated with lower survival rates (approx. 58% after 200 months). These results were statistically significant ($p = 0.007$; Figure 1).

Figures 9, 10 and 11 indicate no statistically significant relationship between count of PDL-1 positive lymphocytes in stroma and tumour and patient survival. Additionally, there is no significant relationship between PD-1 count in stroma and survival (Figure 12). However, Figure 13 shows a significant correlation between the count of PD-1 positive lymphocytes in the tumour and survival ($p = 0.009$), as well as between the combined PD-1 lymphocyte count and survival ($p = 0.017$). The highest significance is found between the low and the high expressing group ($p = 0.009$; Table 5).

3.7 Discussion (500 words max):

This experiment shows a distinct difference in the expression of PDL-1 and PD-1 proteins in early and late stages of colorectal cancer. The effect of this expression differs between stages, as seen here in the difference in survival rates of patients in the two cohorts. In the early stages (stage 1-2) of colorectal cancer, PDL-1 expression does not form a relationship with survival rates. Therefore, PDL-1 cannot be relied on as predictors of survival in this period. Conversely, in later stages (stage 3-4) of colorectal cancer, higher expression of PDL-1 in tumour cells is shown to correlate to a significant degree with higher survival rate in patients. Our results therefore suggest the potential use of PDL-1 as a marker for predicting survival of colorectal cancer patients with stages 3-4.

These results are supported by Droeser et al. (2013), who found that high PDL-1 expression in MMR-proficient colorectal cancer was associated with a favourable prognosis. This study, however, also found a relationship between high expression of PDL-1 in early stages of CRC and positive prognosis – something that our study failed to do. Shiraliyeva et al. (2017) showed clear PDL-1 expression in patients with Hereditary Non-Polyposis Colorectal Cancer (HNPCC), and since it has been found that some patients respond well to PD-1 blockage

they also concluded the usefulness of mapping PDL-1 status in CRC patients. The association between high PDL-1 expression and a positive prognosis has also been found in other cancers, such as rectal adenocarcinoma (Hecht et al., 2016).

Paulsen et al. (2016) found that PDL-1 positive stromal lymphocytes and PD-1 positive tumour infiltrating lymphocytes could be used as prognostic factors in non-small cell lung cancer. This is in accordance with our results, which show a statistically significant relationship between the PD-1 positive lymphocytes in the tumour, as well as the combined PD-1 count, and survival in patients with later stages of CRC. Our results also point to that PD-1 positive lymphocyte count may also play a role in this in the earlier stages of colorectal cancer. However, our results suggest no statistical significance of the PDL-1 positive stromal and tumour infiltrating lymphocytes. Thus, further investigation into the importance of PDL-1 and PD-1 positive immune cells in colorectal cancer is needed.

In conclusion, our results point towards the possibility of using PDL-1 expression in tumour cells and PD-1 positive lymphocytes as predictors of disease-specific survival in colorectal cancer patients. However, more work is needed to be able to validate these results, determine the specificity and functions of the proteins, and later evaluating their responses to immunotherapy. The same goes for other proteins that are being investigated, in this project and in other studies, such as LAG-3 and TIM-3. The results of the importance of LAG-3 and TIM-3 proteins in relationship with patient survival, as initiated in this project, remains to be seen. Further research is essential to explore, determine and develop the specific markers that can be used to predict, diagnose and combat colorectal cancer in the future.

4. Reflection by the student on the experience and value of the studentship (300 words max):

This studentship has provided me with valuable experience and knowledge about research and has introduced me to working in the field. It has increased my interest in immunology and cancer studies and confirmed my intentions of pursuing research in the future.

I was given the opportunity to practice skills acquired at university, as well as to learn novel skills that will be valuable in the future, both in my studies and career. I have learned the essential safety precautions for working in a laboratory, together with common laboratory procedures, such as immunohistochemistry, pH calibration and preparation of solutions of different concentrations. In addition, I have learned new methods of statistical analysis, and have carried them out on big data-sets. Practicing these skills during my studentship has improved my accuracy and confidence in performing them.

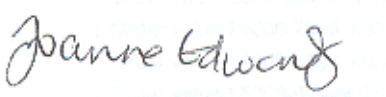
Alongside this material knowledge, I have understood the importance of trusting myself, as well as the value of asking questions. I think I have spotted and solved problems critically, and I have been allowed to learn from my mistakes. The studentship has made me more independent and has fuelled my curiosity for finding and learning new information through research. The experiences of this summer have developed my confidence in multiple ways.

I have experienced the importance of working with something you are passionate about, and I feel lucky to have been involved in such an interesting and innovative area of research with such helpful colleagues. I am fortunate to have the results of my work published in the future, which will prove valuable in my future search for employment. I am happy to have contributed to the knowledge about the involvement of the immune system in colorectal cancer, and I am looking forward to following the important developments of this field in the future.

5. Dissemination: (note any presentations/publications submitted/planned from the work):

The results of this project will be assembled and published by the lab group of Dr Joanne Edwards, the Institute of Cancer Sciences at University of Glasgow.

6. Signatures:



Supervisor Date 20th August 2018

Student Date 20th August 2018

7. References

Droeser, R., Hirt, C., Viehl, C., Frey, D., Nebiker, C., Huber, X., Zlobec, I., Eppenberger-Castori, S., Tzankov, A., Rosso, R., Zuber, M., Muraro, M., Amicarella, F., Cremonesi, E., Heberer, M., Iezzi, G., Lugli, A., Terracciano, L., Sconocchia, G., Oertli, D., Spagnoli, G. and Tornillo, L. (2013). Clinical impact of programmed cell death ligand 1 expression in colorectal cancer. European Journal of Cancer, 49(9), pp.2233-2242.

Hecht, M., Büttner-Herold, M., Erlenbach-Wünsch, K., Haderlein, M., Croner, R., Grützmann, R., Hartmann, A., Fietkau, R. and Distel, L. (2016). PDL-1 is upregulated by radiochemotherapy in rectal adenocarcinoma patients and associated with a favourable prognosis. *European Journal of Cancer*, 65, pp.52-60.

Paulsen, E., Kilvaer, T., Khanehkenari, M., Al-Saad, S., Hald, S., Andersen, S., Richardsen, E., Ness, N., Busund, L., Bremnes, R. and Donnem, T. (2017). Assessing PDL-1 and PD-1 in Non–Small Cell Lung Cancer: A Novel Immunoscore Approach. *Clinical Lung Cancer*, 18(2), pp.220-233.e8.

Shiraliyeva, N., Friedrichs, J., Buettner, R. and Friedrichs, N. (2017). PDL-1 expression in HNPCC-associated colorectal cancer. Pathology - Research and Practice, 213(12), pp.1552-1555.

8. Appendix

DM-APA-CRC (diagnosed colorectal cancer stages 1-2)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
PDL1_cytoplasm_whs	123	75.00	240.00	184.0379	26.03199
PDL1_lymph_stroma	111	.00	26.50	8.5450	5.51430
PDL1_lymph_tumour	111	.00	9.33	1.6186	1.43167
PD1_lymph_stroma	109	.00	26.00	7.0398	5.33429
PD1_lymph_tumour	109	.00	19.00	2.4709	3.65809
Valid N (listwise)	101				

Table 1: Descriptive statistics of collected scores from DM cohort, showing number of patients (N), minimum, maximum and mean values of protein expression (weighted histoscore WHS and positive lymphocyte count), and the standard deviation. Data from DM-CRC-TMA cohort.

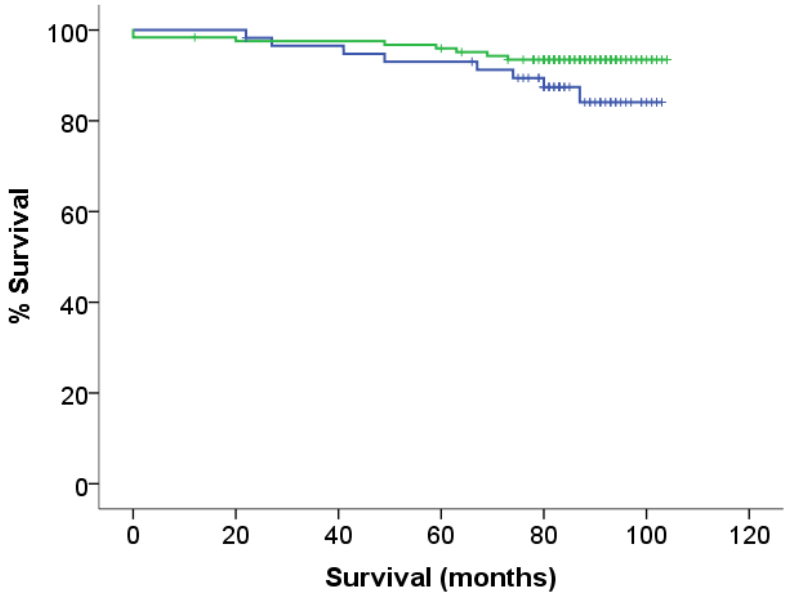


Figure 1: The survival rate (%) of patients with high (green) and low (blue) expression of PDL-1 in cytoplasm of tumour cells. Cut-off point at WHS = 186 (generated using ROC curve) to define high (WHS > 100) and low (WHS < 100) expression. N (total) = 182; n (high) = 124; n (low) = 58. P-value = 0.100. Data from DM-CRC-TMA cohort.

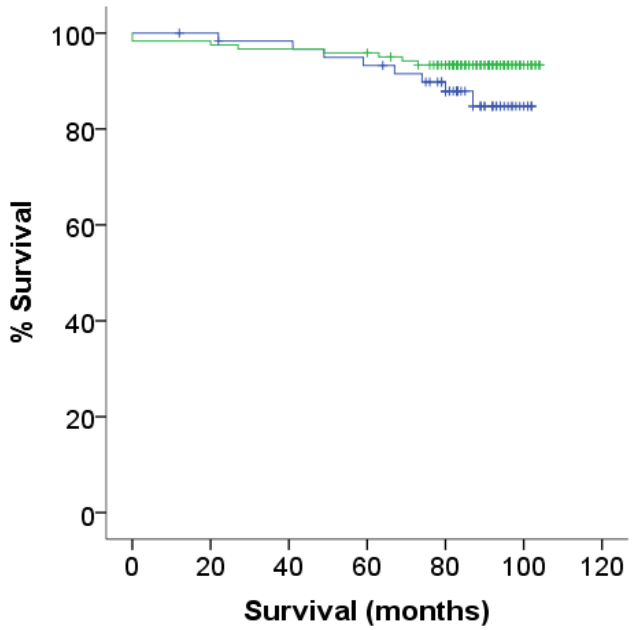


Figure 2: The survival rate (%) of patients with high (green) and low (blue) count of PDL-1 positive lymphocytes in stroma. Cut-off point at n = 8 (generated using ROC curve) to define high (n > 8) and low (n < 8) expression. N (total) = 182; n (high) = 121; n (low) = 61. P-value = 0.133. Data from DM-CRC-TMA cohort.

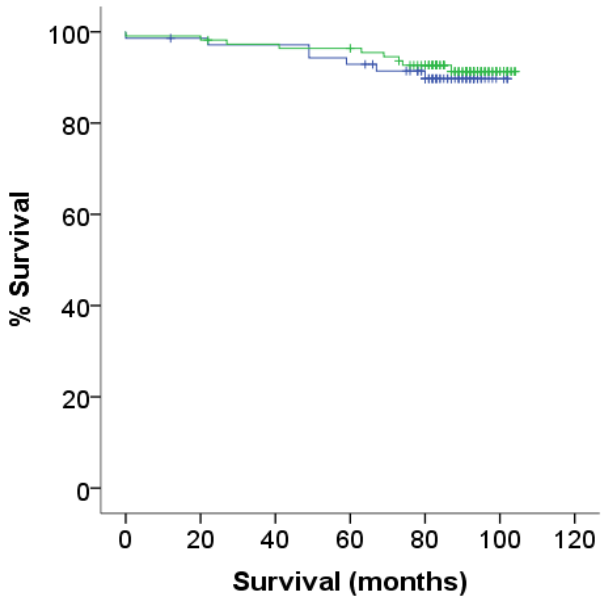


Figure 3: The survival rate (%) of patients with high (green) and low (blue) count of PDL-1 positive lymphocytes in tumour. Cut-off point at n = 1.8 (generated using ROC curve) to define high (n > 1.8) and low (n < 1.8) expression. N (total) = 182; n (high) = 111; n (low) = 71. P-value = 0.635. Data from DM-CRC-TMA cohort.

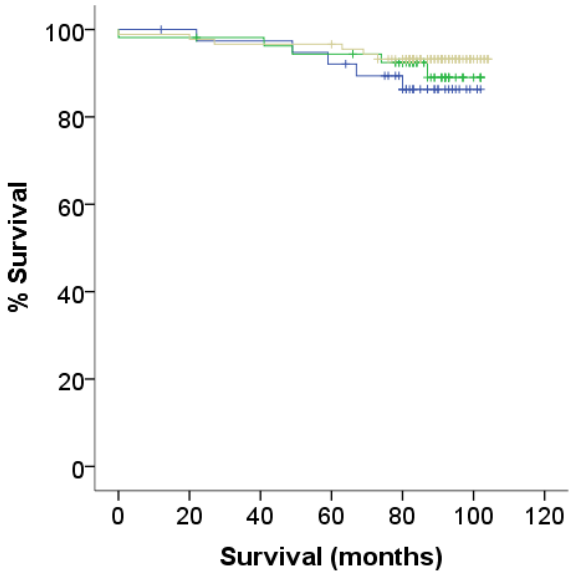


Figure 4: The survival rate (%) of patients with high (yellow), medium (green) and low (blue) combined count of lymphocytes expressing PDL-1 in stroma and tumour (cut-offs used in Figure 2 and 3). N (total) = 182; n (low) = 39; n (medium) = 54; n (high) = 89. P- value = 0.491. Data from DM-CRC-TMA cohort.

Pairwise Comparisons

		.00		1.00		2.00	
	combined_pdl1_code	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank (Mantel-Cox)	.00			.330	.566	1.388	.239
	1.00	.330	.566			.338	.561
	2.00	1.388	.239	.338	.561		

Table 2: Pairwise comparison of statistics (chi square and statistical significance) for combined PDL-1 lymphocyte scores at different levels of expression. Data from DM-CRC-TMA cohort.

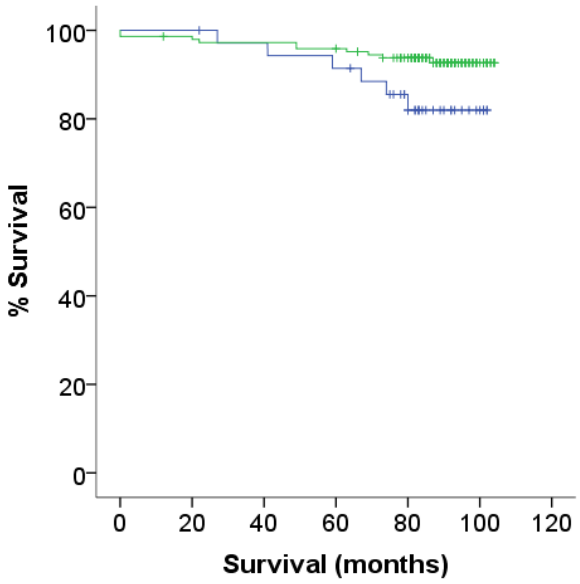


Figure 5: The survival rate (%) of patients with high (green) and low (blue) count of PD-1 positive lymphocytes in stroma. Cut-off point at n = 4.1 (generated using ROC curve) to define high (n > 4.1) and low (n < 4.1) expression. N (total) = 182; n (high) = 146; n (low) = 36. P-value = 0.53. Data from DM-CRC-TMA cohort.

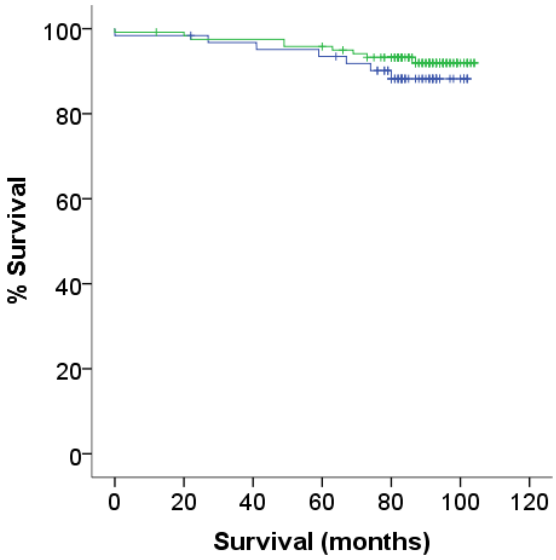


Figure 6: The survival rate (%) of patients with high (green) and low (blue) count of PD-1 positive lymphocytes in tumour. Cut-off point at n = 1.4 (generated using ROC curve) to define high (n > 1.4) and low (n < 1.4) expression. N (total) = 182; n (high) = 120; n (low) = 62. P-value = 0.370. Data from DM-CRC-TMA cohort.

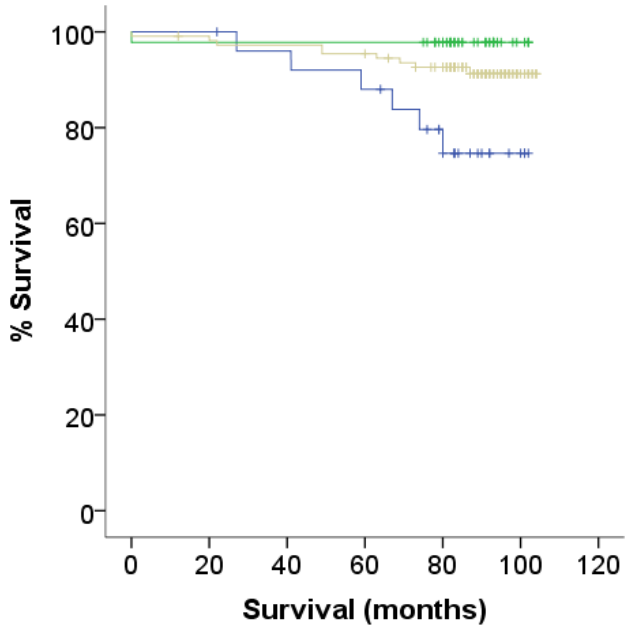


Figure 7: The survival rate (%) of patients with high (yellow), medium (green) and low (blue) combined count of lymphocytes expressing PD-1 in stroma and tumour (cut-offs used in Figure 5 and 6). N (total) = 182; n (high) = 110; n (medium) = 46; n (low) = 26. P-value = 0.007. Data from DM-CRC-TMA cohort.

Pairwise Comparisons

		.00		1.00		2.00	
	combined_pd1_code	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank (Mantel-Cox)	.00			8.748	.003	5.319	.021
	1.00	8.748	.003			1.829	.176
	2.00	5.319	.021	1.829	.176		

Table 3: Pairwise comparison of statistics (values for chi square and statistical significance) for combined PD-1 scores at different levels of expression. Data from DM-CRC-TMA cohort.

AP-CRC-TMA (diagnosed colorectal cancer stages 3-4)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
PDL1_cyto_whs	560	.00	230.00	121.7969	32.74994
PD1_lymph_count_stroma	567	.00	40.33	3.0547	4.20590
PD1_lymph_count_tumour	570	.00	19.33	1.4393	2.75707
PDL1_lymph_count_stroma	553	.00	24.75	5.5121	5.22278
PDL1_lymph_count_tumour	564	.00	27.75	2.2368	2.11038
Valid N (listwise)	512				

Table 4: Descriptive statistics of collected scores from AP cohort, showing number of patients (N), minimum, maximum and mean values of protein expression (weighted histoscore (WHS) and positive lymphocyte count), and the standard deviation. Data from AP-CRC-TMA cohort.

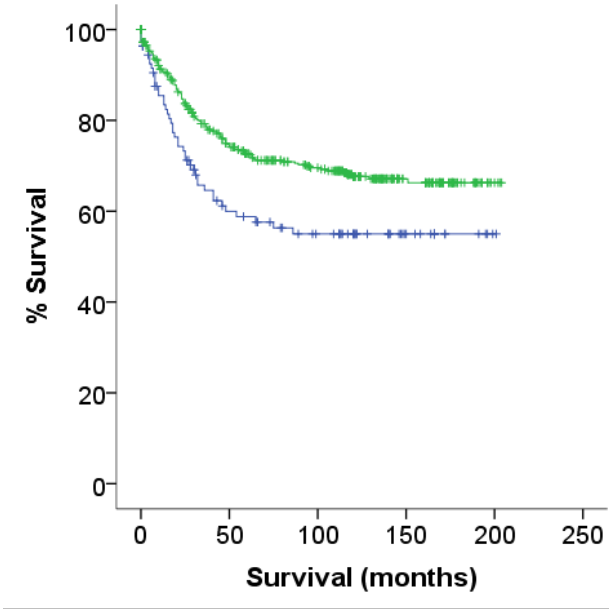


Figure 8: The survival rate (%) of patients with high (green) and low (blue) expression of PDL-1 in cytoplasm of tumour cells. Cut-off point at WHS = 100 (generated using ROC curve) to define high (WHS > 100) vs low (WHS < 100) expression. N (total) = 544; n (high) =433; n (low) = 111. P-value = 0.007. Data from AP-CRC-TMA cohort.

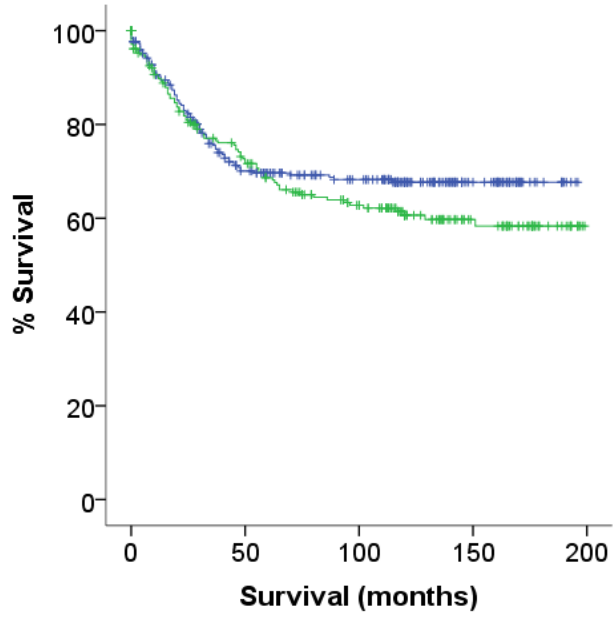


Figure 9: The survival rate (%) of patients with high (green) and low (blue) count of lymphocytes expressing PDL-1 in stroma. Cut-off point n = 4.5 (generated using ROC curve) to define high (>4.5) vs low (<4.5) expression. N (total) = 538; n (high) = 237; n (low) = 301. P-value = 0.198. Data from AP-CRC-TMA cohort.

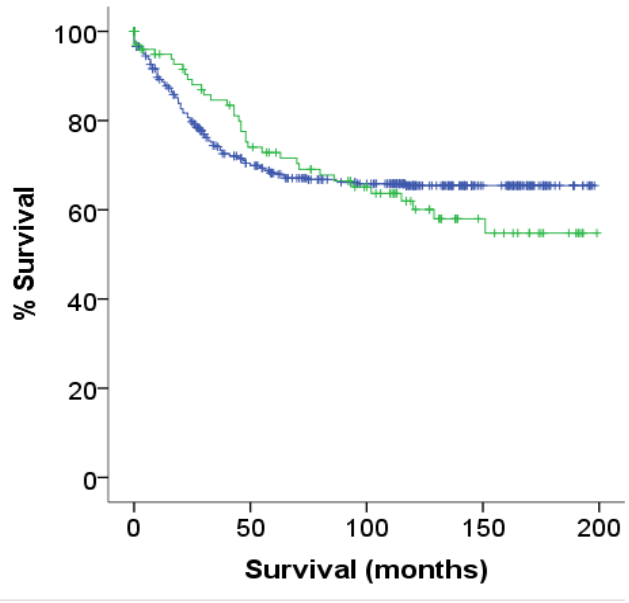


Figure 10: The survival rate (%) of patients with high (green) and low (blue) count of lymphocytes expressing PDL-1 in tumour. Cut-off point n = 3.5 (generated using ROC curve) to define high (>3.5) vs low (<3.5) expression. N (total) = 549; n (high) = 101; n (low) = 448. P-value = 0.713. Data from AP-CRC-TMA cohort.

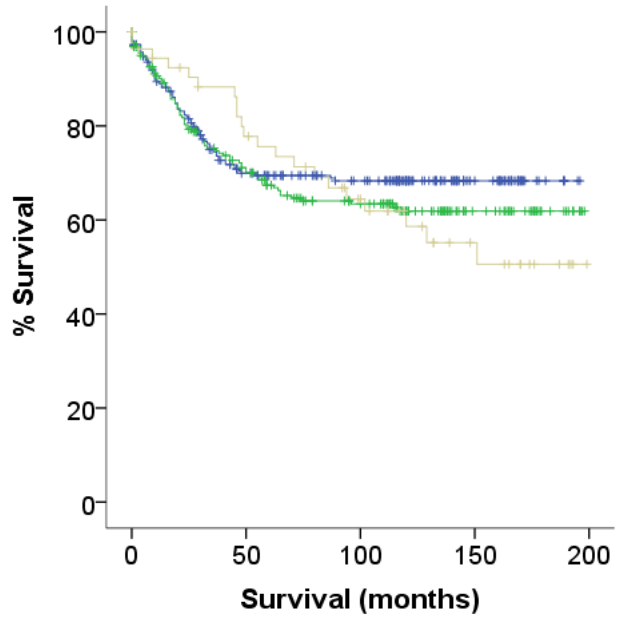


Figure 11: The survival rate (%) of patients with high (yellow), medium (green) and low (blue) combined count of lymphocytes expressing PDL-1 in stroma and tumour (cut-offs used in Figure 9 and 10). N (total) = 538; n (high) = 55; n (medium) = 227; n (low) = 256. P-value = 0.535. Data from AP-CRC-TMA.

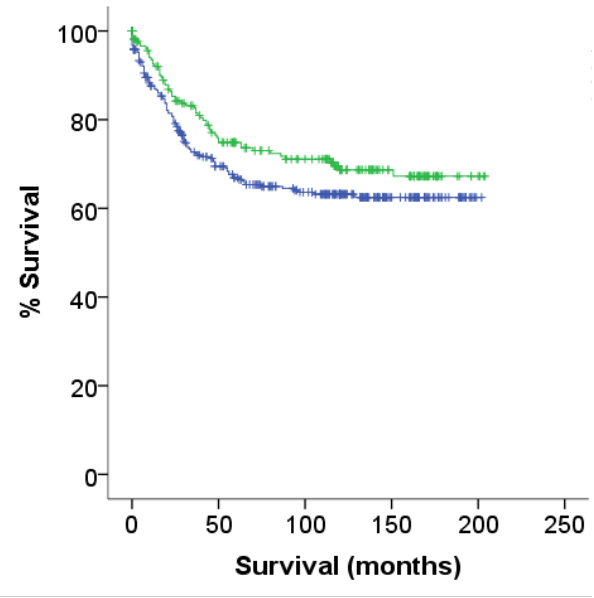


Figure 12: The survival rate (%) of patients with high (green) and low (blue) count of lymphocytes expressing PD-1 in stroma. Cut-off point n = 2.5 (generated using ROC curve) to define high (>2.5) vs low (<2.5) expression. N (total) = 551; n (high) = 213; n (low) = 338. P-value = 0.103. Data from AP-CRC-TMA cohort.

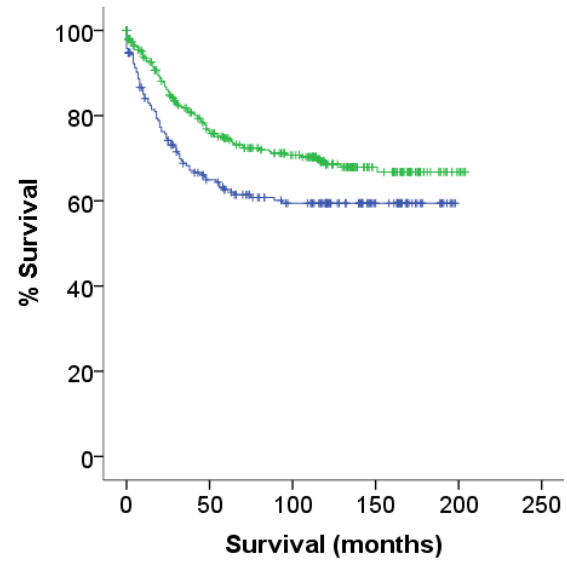


Figure 13: The survival rate (%) of patients with high (green) vs low (blue) count of lymphocytes expressing PD-1 in tumour. Cut-off point defined by absence or presence of positive lymphocytes (generated using ROC curve) expressed as high (presence) vs low (absence) expression. N (total) = 554; n (high) = 342; n (low) = 212. P-value = 0.009. Data from AP-CRC-TMA cohort.

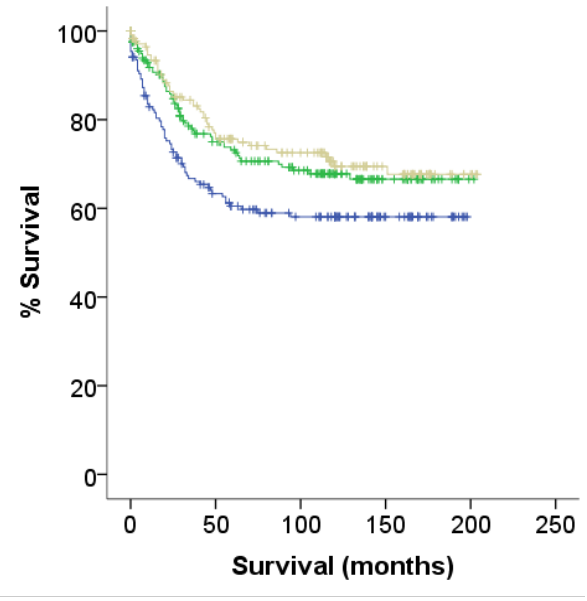


Figure 14: The survival rate (%) of patients with high (yellow), medium (green) and low (blue) combined count of lymphocytes expressing PD-1 in stroma and tumour (cut-offs used in Figure 12 and 13). N (total) = 551; n (high) = 175; n (medium) = 204; n (low) = 172. P-value = 0.017. Data from AP-CRC-TMA cohort.

Pairwise Comparisons

		.00		1.00		2.00	
	combined_pd1_code	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank (Mantel-Cox)	.00			4.701	.030	6.786	.009
	1.00	4.701	.030			.349	.555
	2.00	6.786	.009	.349	.555		

Table 5: Pairwise comparison of statistics (values for chi square and statistical significance) for combined PD-1 scores at different levels of expression. Data from AP-CRC-TMA cohort.