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: Loi Forename: Lynette

   E-mail address: 2172880L@student.gla.ac.uk

2. Supervisor

   Surname: Quinn Forename: Jean

   E-mail address: jean.quinn@glasgow.ac.uk

3. Research Project Report

3.1 Project Title (maximum 20 words):

Role of TAK1 in the development and progression of colorectal cancer.

3.2 Project Lay Summary (copied from application):

Colorectal cancer is one of the most common types of cancer. It is the third most common cancer in men and the second most common in women.
It is being increasingly recognized that an inflammatory response is an important factor associated with colorectal patient outcome and survival. In this project we will investigate what links inflammation with tumour growth and the environment surrounding the tumour. We will assess expression of key proteins in human colorectal tumours see if these are linked to inflammation associated with the tumour or clinical outcome measures such as tumour grade and patient survival.

3.3 Start Date: 26th June 2017 **Finish Date:** 4th August 2017

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

The aim of the current project is investigate the clinical relevance and establish if there is a link between systemic inflammation, local inflammation, and TAK1 in human colorectal tumours.

The objective is therefore to assess the association of TAK1 protein expression in a cohort of 1035 colorectal tumours with systemic, local inflammation, measures of the tumour microenvironment and patient survival.

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

Immunohistochemistry (IHC) was employed to stain tissue microarrays (TMA) prepared using specimens from 1035 patients with colorectal cancer. Antibodies against TAK1 were used. The stained TMAs were scored manually using weighted histoscore.

Due to time constraint and some technical difficulties, the slides prepared were not used for this report. Data was employed from another cohort of 221 patients for statistical analysis instead. Association between survival and patients phenotypes was determined using SPSS. SPSS was also used to determine whether significant associations exist between TAK1 expression and systemic and local inflammation, other clinical phenotypes such as patient survival, expression of other protein including BRAF, MMR status, age, sex and tumour location.

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

TAK1 data was included from 221 patients. The level of TAK1 expression distribution is shown in figure 1. Figure 2 and 3 show low and high expression of TAK1 on tumours stained using IHC.

<u>Association of TAK1 expression with patient's survival</u>

From the 221 patients, no significant difference in expression and survival was observed ($p=0.757$) as shown in figure 4 and table 2.

<u>Association of TAK1 expression with survival in relation to tumour location</u>

From the 221 patients with expression available, 76 patients had tumour on the right side, 66 on the left side and 79 on the rectum.

For patients with right sided tumour, there is no significant difference in expression and survival (p=0.525) as shown in figure 5 and table 3.

For patients with left sided tumour, there is no significant difference in expression and survival (p=0.353) as shown in figure 6 and table 4.

For patients with tumour on the rectum, there is no significant difference in expression and survival (p=0.536) as shown in figure 7 and table 5.

Association of TAK1 expression with survival in relation to MMR status

From the 210 patients with expression available, 179 was MMR competent and 31 was MMR deficient.

For MMR competent patients, no significant difference in expression and survival was observed (p=0.893) as shown in figure 8 and table 6.

For MMR deficient patients, no significant difference in expression and survival was observed (p=0.223) as shown in figure 9 and table 7.

Association of TAK1 expression with survival in relation to BRAF status

From the 199 patients with expression available, 150 was BRAF negative and 49 was BRAF positive.

BRAF negative patients showed no significant difference in expression and survival (p=0.626) as shown in figure 10 and table 8.

For BRAF positive patients, no significant difference in expression and survival was observed (p=0.345) as shown in figure 11 and table 9.

Chi square test

In conclusion, TAK1 has no association with clinical parameters as shown in table 10 and no association with inflammation or tumour microenvironment as shown in table 11.

3.7 Discussion (500 words max):

Transforming growth factor B activated kinase 1 (TAK1) is an upstream of the inhibitory kappa kinases (IKK) which activates the NF-kB pathway. Once it is activated, tumour shows anti-apoptotic trait. NF-kB activation is mainly driven by inflammatory cytokines within the tumour environment[1]. Therefore, TAK1 is known to be associated with inflammatory signalling and proposed to form the link between tumour progression and inflammation. Studies have shown significant anti-inflammatory and anti-tumour activities in animal models when selective chemical TAK1 inhibitors were administered[2]. TAK1 inhibition has been identified as a potential therapeutic strategy in KRAS-dependent colon cancer[3]. In addition, another study has shown the role of TAK1 in activation of p45-IKK (independent of NF-kB) in BRAF-mutated cancer cells which could be therapeutically exploited[4].

This project aimed to investigate the association of TAK1 to local and systemic inflammation and the outcome of colorectal cancer patients. It is well established that systemic inflammation is associated with poor prognosis in colorectal cancer patient but local inflammation is associated with good prognosis. However, the signalling pathways that link inflammation to progression of tumour are not well understood.

The result of this project shows level of TAK1 expression has no signification association with local and systemic inflammation as well as patient's survival. As this statistical analysis only involved 221 patients, another study which involves data from more patients could be conducted in order to increase the power of the result of the study. Unfortunately, due to time constraint, the statistical analysis of 1035 patients could not be completed within the time allocated for this studentship.

In conclusion, expression of TAK1 is not statistically significant to be associated with inflammation and prognostic marker for colorectal cancer but a further research looking into expression of phosphorylated TAK1, which is the activated TAK1 should be considered to establish the findings.

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

I am grateful for the opportunity to undertake this studentship. It has definitely contributed to my personal and professional development. It gave me a good insight into the research world and I have also acquired new skills that I would not have learned as part of my medical curriculum. Over the span of 6 weeks, I have learned lab skills such as immunohistochemistry staining and also statistical data analysis.

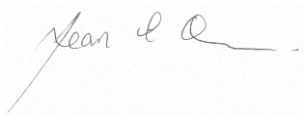
After I was given clear instructions, I was allowed to perform the immunohistochemistry staining independently. In the beginning, I was not confident as I have little to no experience working in a lab setting. It gave me a strong sense of responsibility. When I was staining my first batch of TMAs, the result turned out to be flawed. I was upset because I have not encountered the same problem while staining the practice slides. It might be due to my careless mistake or technical difficulties. I was reassured by my supervisor and I was allowed to carry out more practice slides before staining the second batch of TMAs. This has definitely taught me to pay more attention to tiny details, to be more flexible with changes and most importantly, to persevere. I was relieved that the second batch turned out to be ideal.

This is my first exposure to research work and I am glad it is a productive and valuable experience. I have also gained greater appreciation to the contribution of laboratory research to clinical practice.

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

6. **Signatures:**

Supervisor **Date 4/8/17**



Student **Date 3/8/2017**

References :

1. DiDonato, J., Mercurio, F. and Karin, M. (2012). NF-κB and the link between inflammation and cancer. *Immunological Reviews*, 246(1), pp.379-400.
2. Sakurai, H. (2012). Targeting of TAK1 in inflammatory disorders and cancer. Trends in Pharmacological Sciences, 33(10), pp.522-530.
3. Singh, A., Sweeney, M., Yu, M., Burger, A., Greninger, P., Benes, C., Haber, D. and Settleman, J. (2012). TAK1 Inhibition Promotes Apoptosis in KRAS-Dependent Colon Cancers. Cell, 148(4), pp.639-650.
4. Margalef, P., Colomer, C., Villanueva, A., Montagut, C., Iglesias, M., Bellosillo, B., Salazar, R., Martinez-Iniesta, M., Bigas, A. and Espinosa, L. (2015). BRAF-induced tumorigenesis is IKK -dependent but NF- B-independent. *Science Signaling*, 8(373), pp.ra38-ra38.

Appendix :

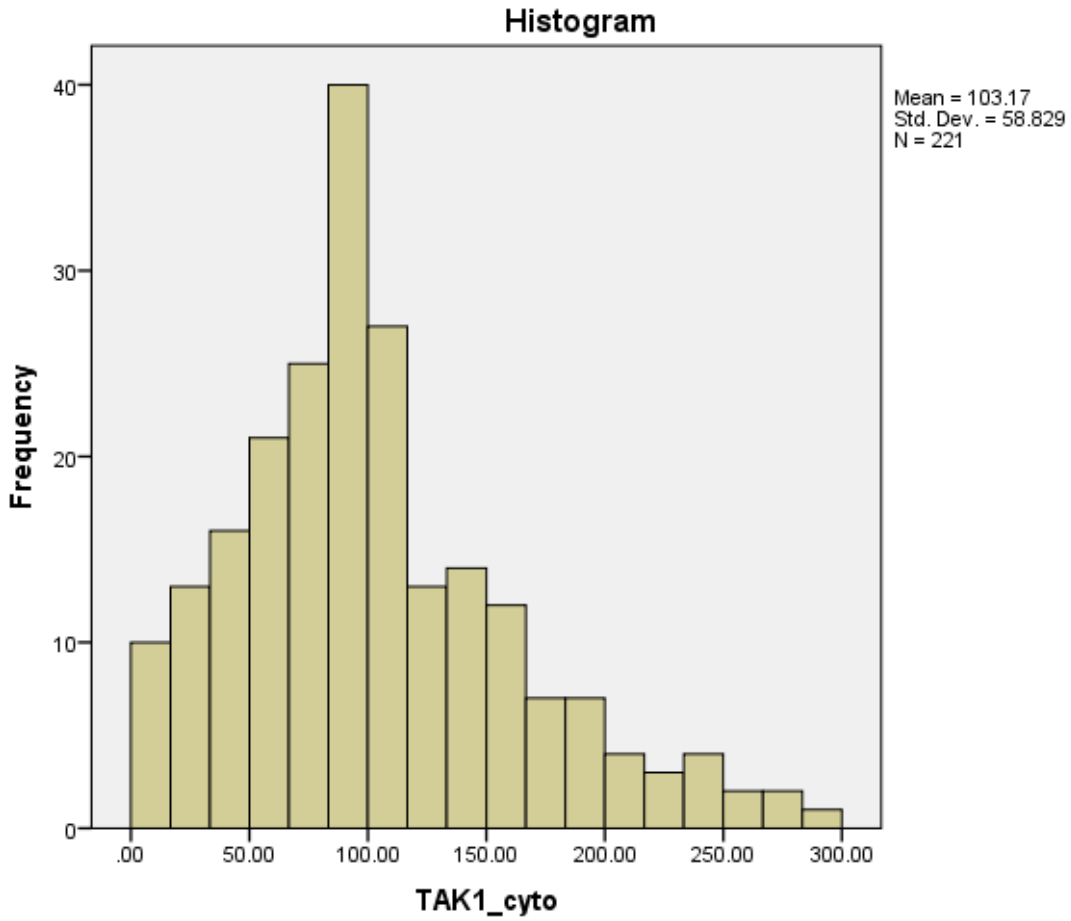


Figure 1 : Histogram shows distribution of 221 patients according to the level of TAK1 expression (0 being lowest and 300 being highest)

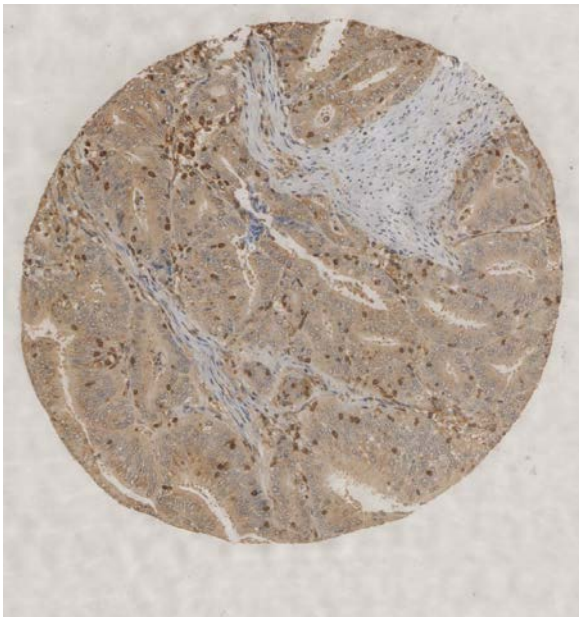


Figure 2 : Low expression of TAK1

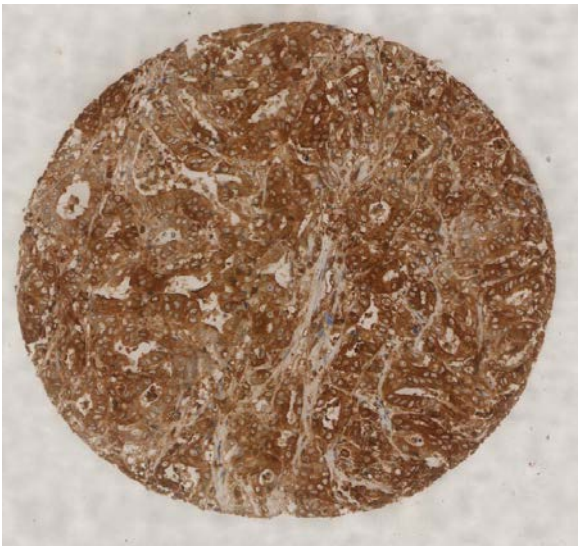


Figure 3 : High expression of TAK1

	p-value
All patients (n=221)	
Sex (female/male)	0.460
Tumour location (right colon/left colon/rectum)	0.765
TNM Stage (I/II/III)	0.001
N-Stage (0/1/2)	0.001
Differentiation (moderate or well/poor)	0.132
Mismatch Repair (MRC/MRD)	0.690
Klintrup-Makinen Grade (strong/weak)	0.006
GMS (0/1/2)	0.002
Immunoscore	<0.001
mGPS (0/1/2)	0.002
Cytoplasmic TAK	0.757

Table 1 : Relationship between patient's parameters and survival

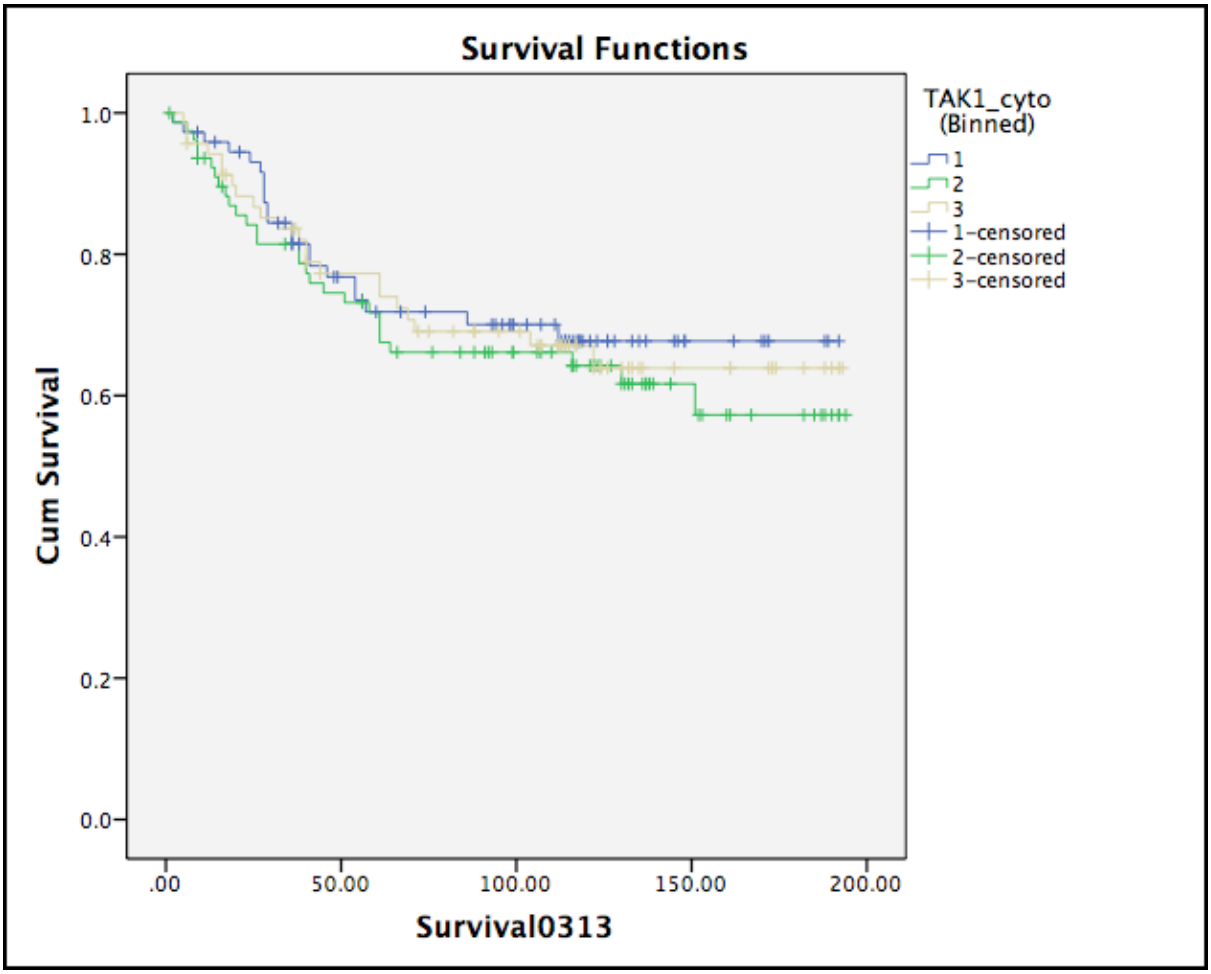


Figure 4 : Relationship between level of TAK1 expression and survival

Expression level of TAK1	No. of patients	Mean survival / months
Low	73	143
Moderate	79	133
High	69	140

Table 2 : Relationship between level of TAK1 expression and survival of the patients

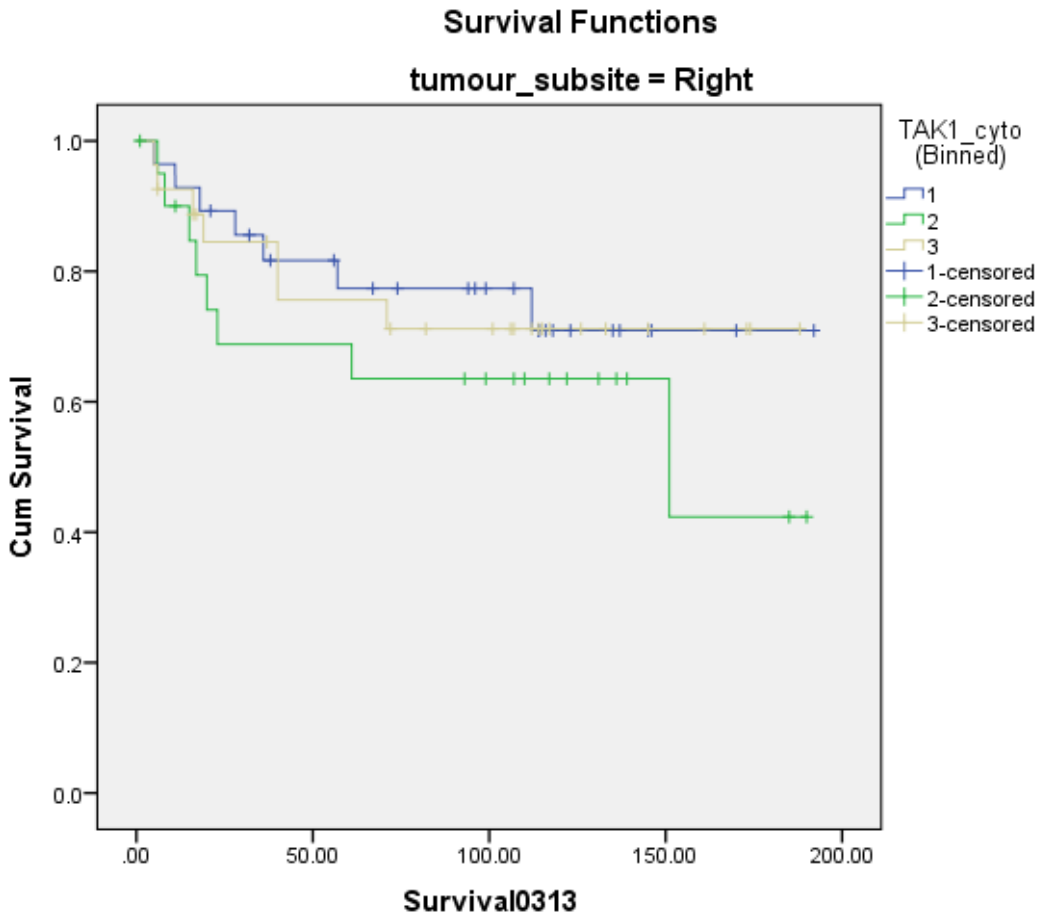


Figure 5 : Relationship between level of TAK1 expression on right tumour and survival

Expression level of TAK1	No. of patients	Mean survival / months
Low	28	149
Moderate	21	120
High	27	142

Table 3 : Relationship between TAK1 expression and survival of patient with right sided tumour.

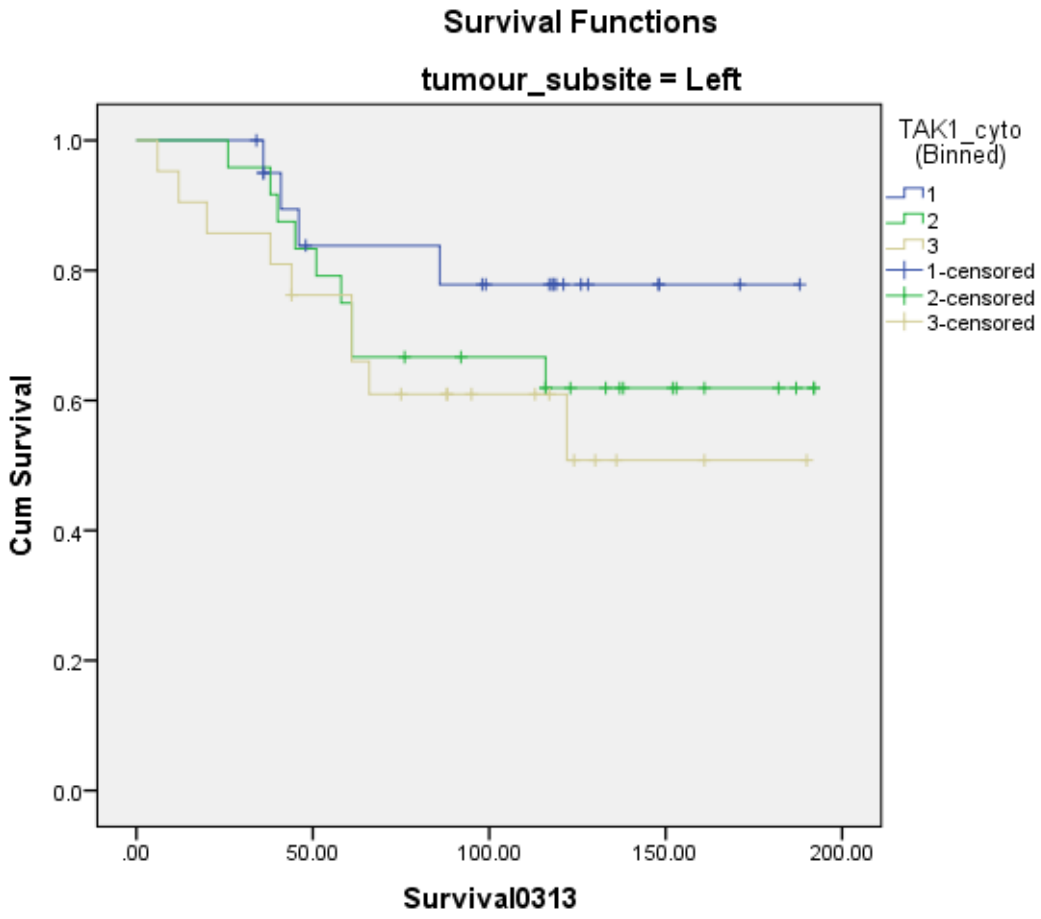


Figure 6 : Relationship between level of TAK1 expression on left tumour and survival

Expression level of TAK1	No. of patients	Mean survival / months
Low	21	158
Moderate	24	140
High	21	124

Table 4 : Relationship between TAK1 expression and survival of patient with left sided tumour.

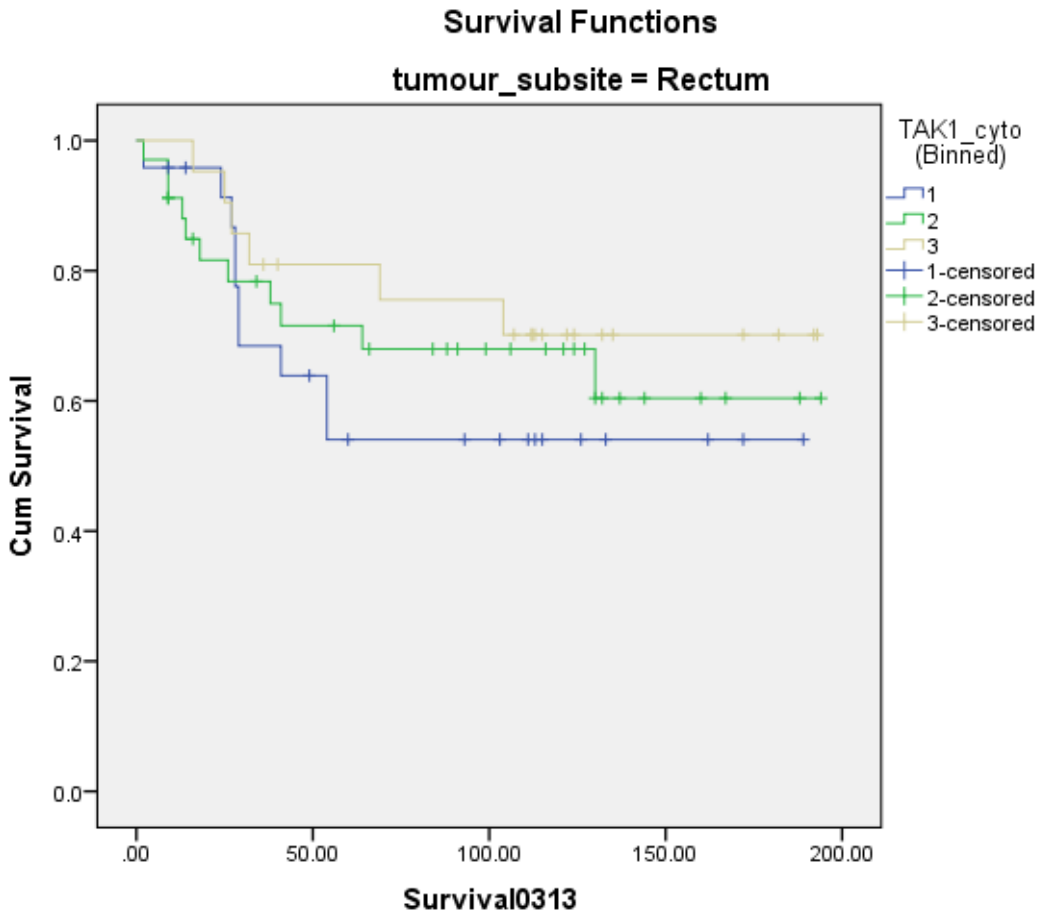


Figure 7 : Relationship between level of TAK1 expression on rectum tumour and survival

Expression level of TAK1	No. of patients	Mean survival / months
Low	24	116
Moderate	34	134
High	21	149

Table 5 : Relationship between TAK1 expression and survival of patient with tumour on rectum.

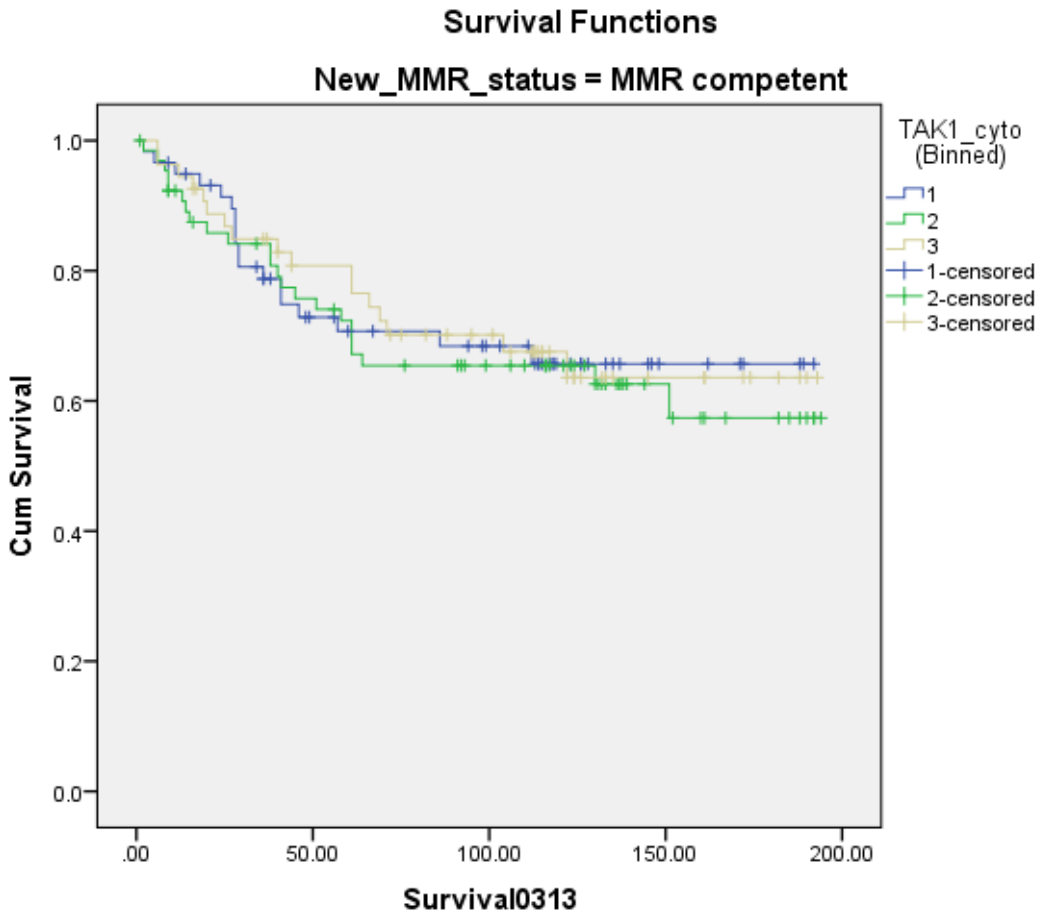


Figure 8 : Relationship between TAK1 expression and survival for MMR competent patients

Expression level of TAK1	No. of patients	Mean survival / months
Low	59	139
Moderate	66	134
High	54	141

Table 6 : Relationship between TAK1 expression and survival for MMR competent patients

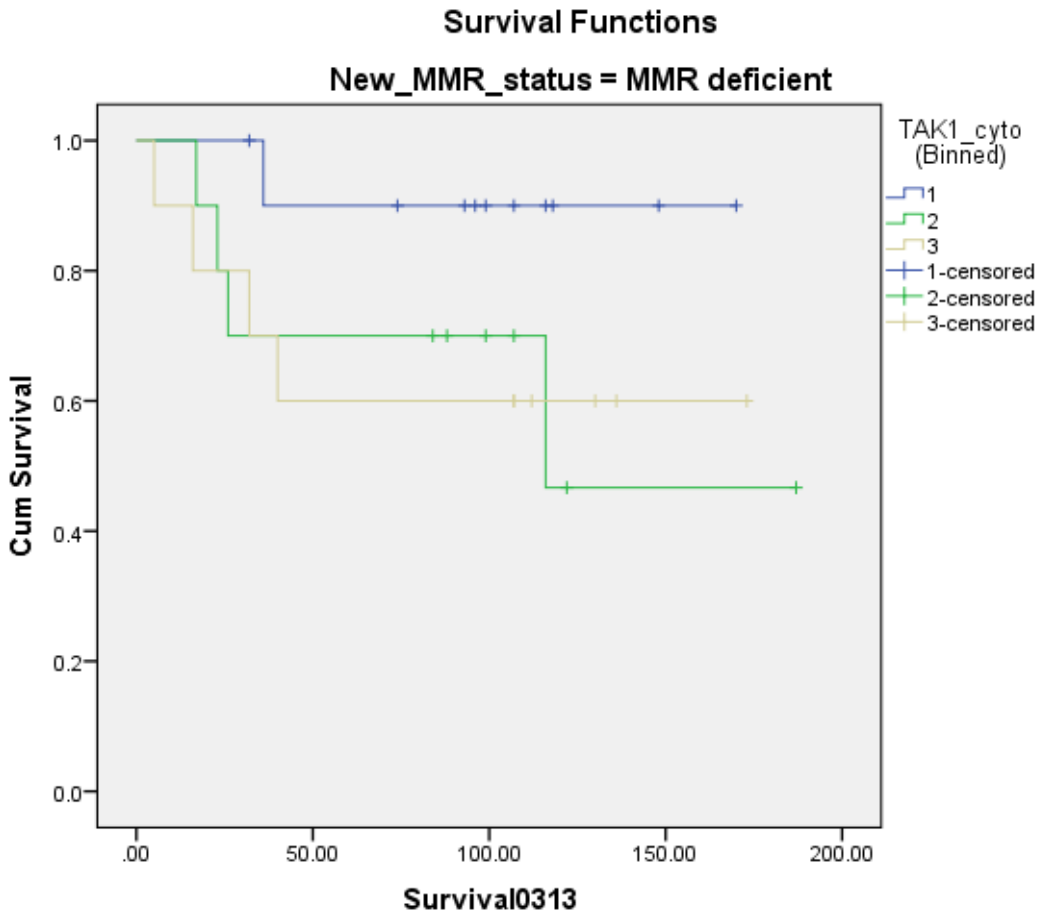


Figure 9 : Relationship between TAK1 expression and survival for MMR deficient patients

Expression level of TAK1	No. of patients	Mean survival / months
Low	11	156
Moderate	10	120
high	10	113

Table 7 : Relationship between TAK1 expression and survival for MMR deficient patients

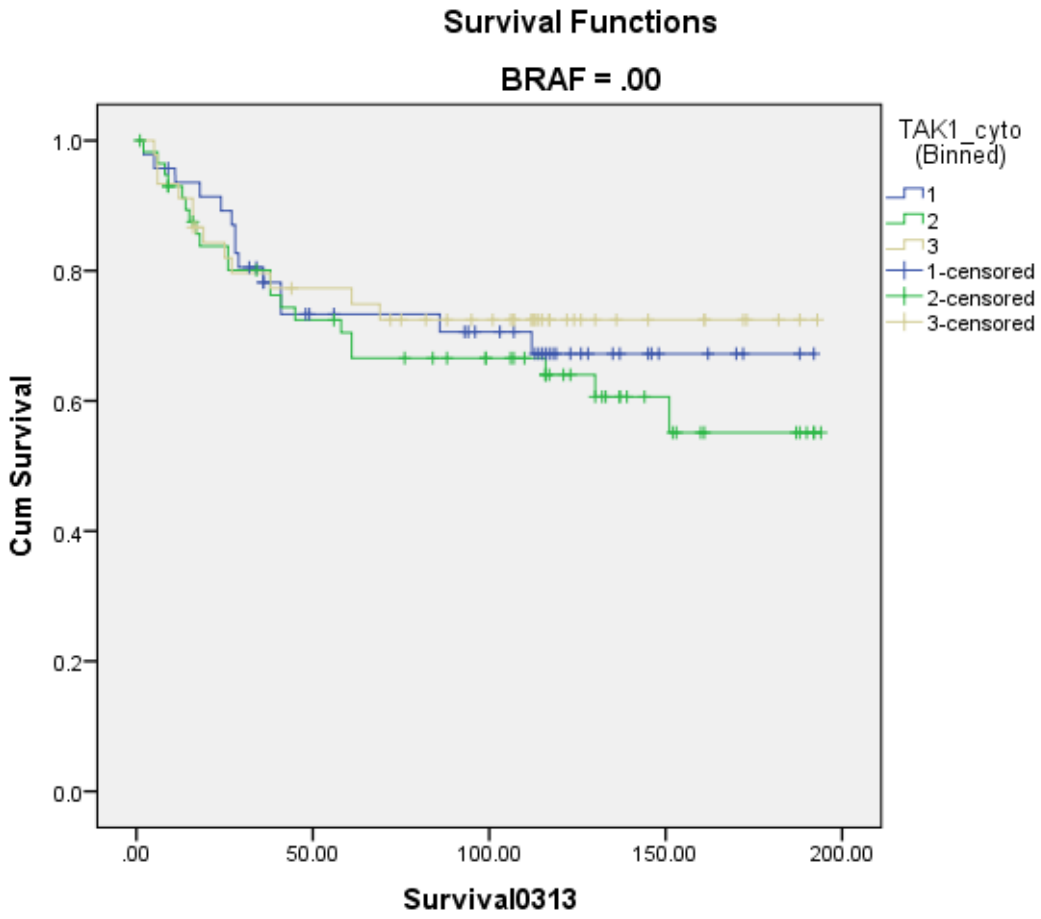


Figure 10 : Relationship between TAK1 expression and survival for BRAF negative patients

Expression level of TAK1	No. of patients	Mean survival / months
Low	47	141
Moderate	58	132
High	45	146

Table 8 : Relationship between TAK1 expression and survival for BRAF negative patients

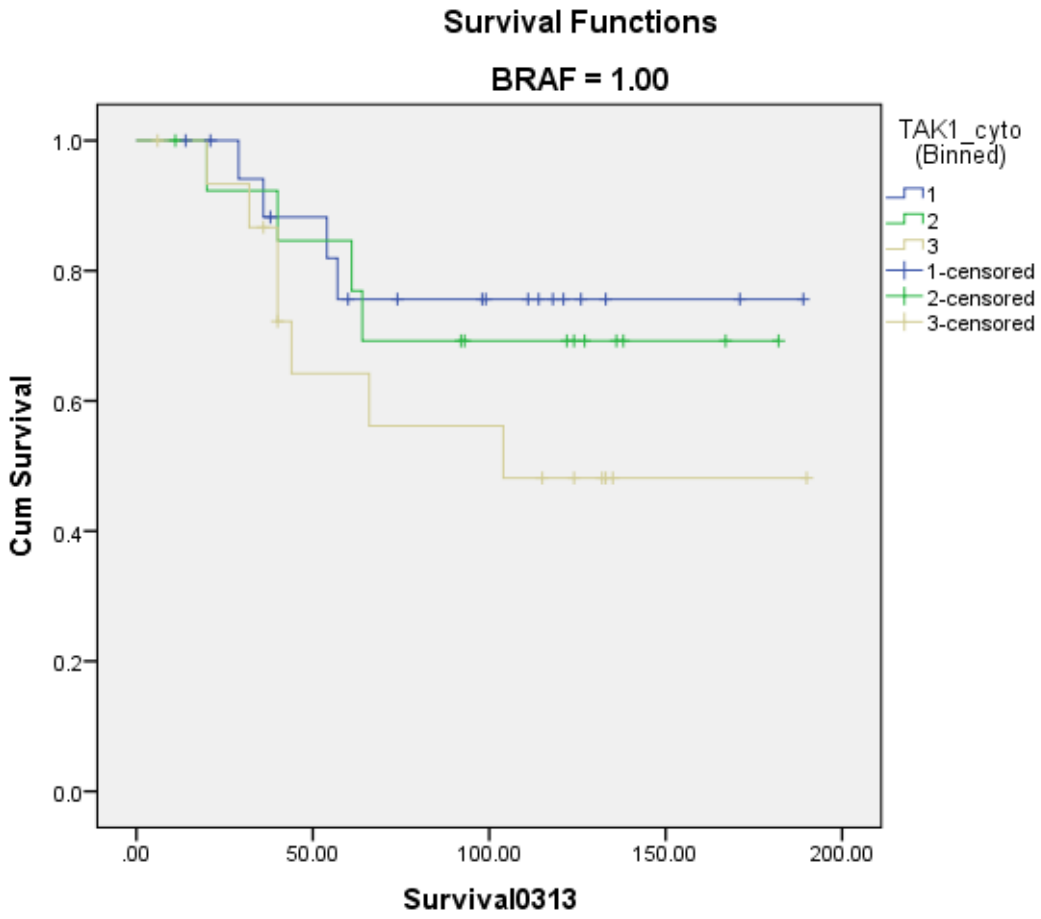


Figure 11 : Relationship between TAK1 expression and survival of BRAF positive patients

Expression level of TAK1	No. of patients	Mean survival / months
Low	19	153
Moderate	14	140
High	16	117

Table 9 : Relationship between TAK1 expression and survival of BRAF positive patients

	1	2	3	p-value
Sex (Female/Male)	27/46	40/39	34/35	0.138
Age (<60/>60)	28/45	31/48	27/42	0.924
Location (Right/left/rectum)	28/21/24	21/24/34	27/21/21	0.846
TNM (A/B/C)	5/41/27	7/32/40	4/37/28	0.639
Differentiation (well,moderate/poor)	64/9	70/9	59/10	0.704
MMR status (competent/deficient)	59/11	66/10	54/10	0.978
BRAF (negative/positive)	47/19	58/14	45/16	0.716

Table 10 : Association between TAK1 expression and clinical parameters (1 = weak, 2 = moderate, 3 = strong expression level)

	1	2	3	p-value
mgps (0/1/2)	35/30/8	49/20/10	47/16/6	0.051
Klintrup category (strong/weak)	27/46	24/54	24/45	0.772
Stroma area > 50% (no/yes)	49/15	55/19	46/16	0.758
Immunoscore (0/1/2/3/4)	28/8/15/7/8	28/10/11/12/12	24/8/15/6/8	0.791

Table 11 : Association between TAK1 expression with inflammation and tumour microenvironment