Author's response to reviews

Title: The biography of the immune system and the control of cancer: from St Peregrine to contemporary vaccination strategies

Authors:

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Author's response to reviews: see over
Dear editor,

We are most grateful for the reviewers’ comments and their valuable and encouraging suggestions for improvement of our manuscript ‘The biography of the immune system and the control of cancer: from St. Peregrine to contemporary vaccination strategies’

Answers to specific points of reviewer Robert Andtbacka:

**Point 1, major compulsory**
Where the manuscript falls short is in the section titled “The FEBIM studies”. This section and subsequent sections are very misleading to the readership, since they do not discuss other risk factor for developing melanoma: ultraviolet exposure, skin type, genetic predisposition, etc. It would be very beneficial to the readership if the authors discussed the infections and vaccinations and risk of melanoma in the context of other risk factors to provide a more complete and balanced presentation of melanoma risks.

**Answer:**
The section ‘The FEBIM studies’ has been rewritten, extended and split into 2 sections -
The first is entitled ‘Failed immune stimulation’ as melanoma risk factor and its interplay with other risk factors’, and the second is entitled ‘Interpretations from the FEBIM study’. In the first we give a more detailed description, supported by 3 tables, of the joint analyses between the diverse melanoma risk factors and ‘not vaccinated with BCG and/or vaccinia’. In the second new section the interpretations that had been drawn from the joint analyses are re-evaluated. Most relevant are the views on interaction between ‘not vaccinated’ with indicators of UV exposure and the view on interaction with genetic predisposing factors.

**Point 2, minor Essential Revision:**
• Page 11 and Figure 2: Were the patients with clinically node negative disease in the presented study staged with sentinel lymph node biopsy. If not, how did this impact on survival?

**Answer:**
Sentinal biopsy technique had nor been introduced as routine practice at the time of the field phase of the FEBIM study.

**Point 3, minor Essential Revision:**
• Figure 2: Were the patients stratified by stage? If not, how did the authors ensure that the different patient groups were equivalent with the exception of vaccination status? Please provide a detailed explanation for the readership.

**Answer:**
Cases of melanoma in situ were excluded. A further stratification of the melanoma patients was performed for several prognostic factors including tumour thickness (according to Breslow) in a multivariate analysis and this is stated in the revised text –

‘Importantly, the differences shown in Fig. 2 persisted after adjustment for several prognostic factors in a multivariable analysis.’
Answers to specific points of reviewer Ulrich Abel Reviewer’s report:

Point General Remarks a
I suggest that references to overviews be replaced or supplemented with original literature.

Answer:
Done (although this increases the number of cited references).

Point General Remarks b
In many places the manuscript refers to studies and trials without giving any details regarding, e.g., the design and conduct of the studies, the units of observations (e.g. cases, controls), and the sample sizes.

Answer:
Our topic is ‘prevention of melanoma/cancer by infections/ vaccinations. The fields associated with this topic, i.e. spontaneous regression, treatment of cancer with bacterial derivatives, post-operative infections cannot be extended more than already done without greatly extending the length of this paper. We agree with the reviewer that more details should be given on the studies that form the basis of our debate. Therefore considerable extensions are made: FEBIM study, melanoma-yellow fever vaccination study, and the FEBIM survival study arm.

Major Compulsory Revisions
1. Background, para 1-2 as well as para 4:
In the literature there are several rather large collections of case reports of spontaneous regression (SR) of cancer, some of which consider the role of infections prior to the SR (e.g. Rohdenburg, 1918; Everson and Cole, 1956,1966; Stephenson et al., (1971; Nauts, 1980; Maurer and Kölmel, 1996). The Reviewer is quite confident that the Krone et al are familiar with this literature. It may be worthwhile having some (more) of those references in the paper. On the other hand, there are numerous fundamental and well-known methodological reasons why collections of case reports of spontaneous regressions (SRs) are neither well-suited to obtain reasonable estimates of the incidence rates of SRs nor to investigate the causes of SRs. More specifically, they do not allow any conclusions regarding the role of infections in SRs beyond the banal statements that SRs do occur in histologically confirmed cancer and that they have been observed after febrile infections. A reservation to this effect should be included in the paper to avoid creating the impression that infections have been shown to induce tumor regressions at any relevant incidence rate.

Answer:
Reservations on the quality of many of the cited studies is expressed in the text by:

Many of the earlier studies did, however, have severe methodological flaws and the results were quite heterogeneous and contradictory.

2. Background, para 3:
There is a vast amount of literature regarding the use of living bacteria or bacterial toxins in cancer patients. There are even some randomized studies (e.g. Kempin et al. (1981, 1983), Tang et al. (1991) on mixed bacterial vaccines (MBV=Coley’s toxins); Isenberg et al. (1994), who used propionibacterium granulosum; a wealth of studies on OK-432). It would be helpful to mention at least a few of those.

3. Background, para 4:
The role of postoperative infections for the prognosis of cancer patients has been investigated in numerous comparative studies. Some of these studies found a better prognosis for patients who had a postoperative infection compared to patients without infections. However, the studies mostly had severe methodological flaws, and the results were quite heterogeneous and contradictory. E.g., for the special case of lung cancer, Takita (1970) and Ruckdeschel et al. (1972) found a positive influence of a postoperative empyema, whereas this relationship remained unclear in the study by Cady and Clifton (1967), and Brohee et al (1977) even found a negative effect of infections on survival. Finally, the matched-pair study conducted by Minasian et al. (1978) did not find any difference in survival between patients with or without empyema. As for colorectal cancer, the results were contradictory as well (e.g. Müller and Regazzoni, 1975; Fucini et al. 1983; Nowacki and Szymendera, 1985), while for head and neck cancer (Jackson and Rice, 1990, Grandis, 1992) and breast cancer (Teucher and Schindler, 1986) a higher risk of recurrence was observed following febrile infections. For malignant melanoma Papachristo and Fortner (1979) did not find any influence of a wound infection of survival of DFS. The reviewer has not followed more recent literature on this particular subject; however, this does not alter the fact that the findings are by no means unequivocal.

While a long paragraph elaborating this topic may not be needed, mentioning just one study in para 4 may give the false impression that this question has been severely under-investigated in the past. Also, the fact that the findings of the studies are contradictory should be mentioned in the paper.

Answer:
The number of references, and comments on them, has been extended and commented in the text, with the added observation:

‘However, the evidence for the effectiveness of the approach remains disputed.’

4. Discussion, Infection and cancer, para 1:
The manuscript refers to the study by de Martel et al. (2012). In this study, population attributable fractions (PAF) for the risk factor “infection” were estimated for the year 2008 based on relative risks observed in epidemiological studies and the prevalence of exposure in different geographical regions. The method used in the paper is not incorrect, but to quote de Martel et al. “The estimate relies on strong causal assumptions and a simplified statistical model”. The results must indeed be interpreted with great caution. (E.g., it is well-known that the sum of the PAFs for different risk factors can exceed 100%). Therefore, the strong wording used by Krone et al. (“were related to”), which insinuates a causal relationship, should be weakened. As for the study on exposure of infants to acute infections and the risk of childhood ALL, this question had previously been investigated in a case-control study by van
Steensel-Moll et al. (1986).

Answer:
The strong wording is weakened and ref. Steensel-Moll included
In particular in the text to:

‘Another, though possibly related, example of an apparent protective environmental effect on cancer is provided by the association of exposure to cattle in the dairy farming industry that apparently protects against several types of cancer, with statistically significant associations for lung, bladder, pancreatic, and oesophageal cancer [48]. The degree of apparent protection is related to the intensity …….’

5. Discussion, Infection and cancer, para 2:
Here, more information on the study in question (Mastrangelo et al, 2005) should be given. This was a nested case-control study which was embedded in a cohort study on self-employed farmers in Italy. The main finding was a trend towards a lower risk with increasing number of dairy cattle. The wording used by Krone et al. (“demonstration that....protects...”, “with statistically significant protection”) which suggests a causal relationship is too apodictic. In epidemiology, risk associations should always be treated as such, and great caution should be used when interpreting the relationship as causal (“protect”). This is especially true for the study in question, where smoking was the only potential confounder that was controlled. Also, there have been earlier investigations suggesting that persons who are exposed to endotoxins at work have a lower cancer risk (see, e.g., the survey published by Enterline et al., 1985). These earlier finding should be mentioned as well.

Answer:
Done and ref. Enterline included.

6. Darwinian medicine, para 1:
To the reviewer’s knowledge the first sentence in this paragraph (“especially...”) is not correct. While cancer incidence rates are mostly higher in developed countries than in developing countries, the latter show a higher secular increase in incidence rates. The study by Maddams et al. to which the second sentence refers did not investigate cancer incidence (as stated in the manuscript) but prevalence rates (excluding skin cancer). One major finding was that the increase in prevalence was particularly strong for prostate cancer, where it was attributed to the impact of early detection (PSA screening) and improvement in survival. Whether or not a relevant increase in overall prevalence rates occurred if prostate cancer is excluded is not stated.

Answer:
The important and valid comment of the reviewer is included in the text:

‘While cancer incidence rates are mostly higher in developed as compared with developing countries, the latter show a higher secular increase in incident rates.’

The study of Maddams and our comments on it have been deleted.

7. Darwinian medicine, para 2,3:
The connection of the two observations (1, a rise in cancer incidence, seen also in
younger people; 2. an increase in the incidence of several classes of diseases associated with chronic inflammation) appears very speculative, at least if it is aimed at creating the impression that there might be a causal relationship. First, it should be noted that for some cancers (stomach cancer) rates have not increased but decreased in the past decades. Second, even in cases where rates have risen there are often very good reasons for the rise (e.g., smoking; for breast cancer: decrease of the number of pregnancies, etc.). The question of interest in the context of this manuscript would be whether, after adjusting for these known factors, any good evidence exists that inflammation has contributed to a cancer incidence. In the reviewer’s opinion, this is doubtful.

Answer:
We considered the pro and con of deleting the whole section on Darwinian medicine. However, this point of view on diseases with a supposed complex aetiology is an important topic of actual discussions and is related to our topic. Therefore we did not delete but commented in the text:

‘although it remains to be determined whether such inflammation contributes to the incidence of cancer incidence or whether it is just a common epiphenomenon.

8. History of infectious diseases and vaccinations and cancer risk, para 1:
There is a wealth of epidemiological studies on infections and cancer risk (at least 11 case-control studies and 2 cohort studies). Mentioning just one appears a little selective. Also, the results regarding the association between childhood infections and cancer risk are heterogeneous. The case-control study conducted by the DKFZ Heidelberg (Abel et al, 1991) did not find any such association for 5 types of carcinomas.

Answer:
The section is shortened and combined with a section at the beginning. More studies are mentioned.

9. History of infectious diseases and vaccinations and cancer risk, para 4:
Reference no 44 is a short overview by Grange and Stanford. As far as the reviewer can tell, Grange and Stanford have not done any study themselves, and their publication does not contain the detailed description of the Finnish study given in the first sentence of the paragraph. The reference given here should be changed. Also, Grange and Stanford express serious concerns regarding the methodological strength of the study done by Häro et al. (“despite admitted weaknesses in the epidemiological data...”). Therefore, the phrasing used in the manuscript pertaining to these results (“In Finland it was shown...”) should be weakened. The same applies to the formulation “led to a reduced risk”, which suggests a causal effect. Such a causal relationship cannot be inferred from an elevated relative risk observed in the study. The statement regarding a possible protective effect of BCG vaccination against leukaemia is rather speculative. It is based on an increase of leukaemia incidence rates in Great Britain from 1911 and 1959, for which, however, alternative explanations are conceivable. Also, it remains open if no similar increases had occurred before 1911. Note that this would need to be excluded, for otherwise the argument given in the paper would not have much strength.
Answer:
The references Grange and Häro were that were exchanged are now corrected. Phrasing concerning the Häro study was weakened and it was stated in the text:

‘… although of course there may be alternative explanations for the rise in the incidence of leukaemia.’

10. History of infectious diseases and vaccinations and cancer risk, para 5:
Though immunological aspects were not the focus of this review, one exception shall be made, namely, regarding a statement (found in this paragraph) on progression of cancer associated with a Th2 drift. Reference no 51, which was given to substantiate this statement, describes a Taiwanese study published by Kuo et al, 2012. In this study, SIRs for cancer in a sample of 530 patients with newly diagnosed tuberculosis were calculated by comparing the incidence rates in this cohort with those from the general population. For all cancers, as well as several cancer sites, significantly increased SIRs were found. The reviewer cannot tell to what extent this finding (if it indeed represents a causal relationship) supports the medical argument given by Krone et al.. However, the finding of the study itself is questionable and is hardly a basis for any inferences because this type of study is the weakest form of epidemiological evidence, given that no adjustment for confounders was possible. Note that in this particular case very strong and plausible confounders exist due to the fact that both tuberculosis and cancer are strongly related to social status and lifestyle.

Answer:
We gave some attention to Th2 polarization. However the study of Kuo and arguments based on it were deleted. We have also added a reference to the contemporary work of Galon on the importance of the qualitative nature of the tumour-infiltrating T-calla and macrophages – the ‘immunoscore’ – on prognosis.

11. The FEBIM studies, para 1,2
Reference no 53 (Seah et al.) appears to be incorrect. It is mentioned twice in connection with melanoma, but this paper does not contain any statement on melanoma.
As for the FEBIM study, to the reviewers knowledge it is actually one single epidemiological case-control study (not really a series of different studies with different protocols, patient samples, etc.), which served as the basis for several separate analyses. Some details of the study design should be added to permit a better understanding what was actually done.

Answer:
The reference Shea was only wrongly cited (an exchange) and is completely deleted. FEBIM is now used only in singular form. Several details are added.

12. The FEBIM studies, last paragraph:
Referring to the case-control study of Hodges-Vazquez et al. and its finding of a non-significant risk association of malignant melanoma with 17D vaccination in a particular subgroup (>= 10 years since vaccination) Krone et al. state that this [finding] “presumably on account of low numbers in this time group and an observational time frame restricted to only 11.5 years, was not statistically significant.” This interpretation may be criticized for two reasons. First, the non-significance of a
study result should not be interpreted in this way, because - while small sample sizes may be the reason - it may equally be due to the simple fact that no true effect exists. Second, and more importantly, the numbers were, in reality, not small at all. The particular subgroup in question contained no less than 105 cases and 950 matched controls. This is a very large subgroup, and, in particular, it is much larger than the corresponding subgroup in the other case-control study (Mastrangelo et al, reference no. 61) quoted by Krone et al., which contained only 3 cases (see Table 4 in reference no. 61).

**Answer:**
This section has been rewritten, taking up the reviewer’s comments and supplying more details.

13. Effect of prior vaccination on the clinical course of melanoma, para 1:
One should mention that the differences shown in Fig. 2 persisted after adjustment for several prognostic factors in a multivariable analysis. This is of importance for the appraisal of the study result, because otherwise it cannot be excluded that the differences between the unadjusted survival curves are merely due to bias from confounding factors. E.g., without the adjustment the following objection could be raised: Vaccination may be statistically related to social status, which is related to the preparedness to participate in early detection programs, which is associated with the tumor stage at first diagnosis of malignant melanoma.

**Answer:**
The section is extended by giving more details and by stating in the text:

‘Importantly, the differences shown in Fig. 2 persisted after adjustment for several prognostic factors in a multivariable analysis.’

14. Conclusions, para 2
The article argues in favor of using or scientifically exploring preventive vaccination strategies (“We therefore open the debate as to whether extensive controlled vaccination studies should be undertaken in patients and/or regions for whom/where they are needed most urgently”). A brief sketch should be given of the potential design of such studies (retrospective or prospective? Cohort study or randomized study? Study populations, follow-up, endpoints, etc) and their feasibility (e.g., minimum duration of follow-up, ethical questions involved in intervention studies, especially randomized ones).

**Minor Essential Revisions**
Discussion, Infection and cancer/History of infectious diseases and vaccinations and cancer risk: These two sections partially address the same questions, as becomes evident from the title and the contents. Pooling the chapters should be considered.

**Answer:**
The end of the debate was rewritten as a discussion and summary and, as suggested, we make a proposal and discussion of a possible study.

Yours sincerely,

Bernd Krone
Co-authors agreed