Reviewer's report

Title: Use of mitogenic cascade blockers for treatment of C-Raf induced lung adenoma in vivo: Cl-1040 strongly reduces growth and improves lung structure

Version: 1 Date: 27 March 2004

Reviewer: Reinhard Wetzker

Reviewer's report:

General

The experimental data reported by Kramer et al. are sound and coherent in themselves. The reviewer suggest acceptance after minor revision.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Fig.1C. The major point raised by the authors is, quote: „an essential role of the signal flow through the mitogenic cascade for Raf induced transformation“. While the data on the MEK inhibitor are consistent with and suggestive of such a connection, they do not ultimately prove this (see point 2 below). The authors have previously convincingly demonstrated Erk activation by BXB-Raf in BXB-Raf mouse lung tissue. The authors should recapitulate that experiment and investigate the effect of CI-1040/BAY 43-9600 in a phospho-erk Western blot with a corresponding Erk loading control.

2. In the discussion part the authors do not comment on a number of experimental results nor on relevant literature data, but instead expand on BMI-1 literature that seems partially irrelevant. The authors should restructure the discussion section and comment on the following issue: CI-1040 but not BAY 43-9600 reduces adenoma formation. The authors argue that BXB-Raf inhibition may be incomplete due to feedback loops impinging on Raf. This is a good thought since similar observations have been made with the c-Raf inhibitor ZM336372. However, recent evidence from transgenic mice argues that MEK may not be a substrate for c-Raf in vivo after all and this has prompted the notion that c-Raf may signal independently of its kinase activity. Indeed, using the same approach as in the present manuscript, the authors have previously shown that wild type c-Raf expression induces lung adenomas in the absence of (detectable) Erk phosphorylation. Thus, Raf (BXB-Raf here) and MEK may fulfill different roles in lung cells and adenoma onset. This provides a second interpretation for the different effects of CI-1040 and BAY 43-9600 and should be commented by the authors. In this regard, the assessment of p-Erk positive cells and its reported reduction by CI-1040 (Fig.1C) is no prove for linear BXB-Raf/MEK/Erk signal flow, since CI-1040 reduces overall cell number as judged from the results presented in Fig.2B,C,D.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. There are some spelling errors (e.g. two faults in the final Conclusion section). Further, there is an inappropriate description of experimental results. In the maintext and in the legend to Figure 1 (B,C) the authors describe the detection of phosphorylated, active Erk in lung sections using antibodies
selective for phospho-Erk as, quote: „the expression of p-Erk“; phosphorylation is a post-translational modification and it is not p-Erk expression. This should be changed.

2. Fig.1A. It is apparent that the IC50 for both inhibitors, as tested in vitro, is in good agreement with previously published data, at least for BAY 43-9006. The authors should state the IC50 value obtained in their experiments.

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential revisions
Level of interest: An article of importance in its field
Quality of written English: Needs some language corrections before being published
Statistical review: No
Declaration of competing interests:
None