Reviewer's report

**Title:** Assessment of the role of transcript for GATA-4 as a marker of unfavorable outcome in human adrenocortical neoplasms

**Version:** 5  **Date:** 9 March 2004

**Reviewer:** Alessandro Peri

**Reviewer’s report:**

The authors improved the quality of the manuscript, by performing dot blot analysis, which increased both sensitivity and specificity of the experimental data. Accordingly, the iconography of the manuscript was improved. A semi-quantitative analysis was also performed and showed that larger amounts of GATA-4 transcript tend to correlate with a more severe outcome.

The distinction between non-metastasizing and metastasizing tumors has been more thoroughly detailed. The authors should add the papers by Gicquel (2001) and Aubert (2002) among the references, in order to strengthen their statement about the criteria for distinguishing adrenocortical malignancy.

Nevertheless, there are some issues that need to be re-evaluated, because it appears that there is discrepancy between some of the statements and the strength of the results. In particular:

Page 11. The authors should not say that GATA-4 expression appears to be inversely correlated to LHR expression (i.e. this is not true in sample # 10, 16, 18, 22b).

Page 11. A cut-off of 1.77 was found to distinguish between NM and MR tumors. However, this value would lead to a false negative diagnosis in 4 out of the 10 MR tumors (# 14, 16, 21, 22a). In addition, which is the intraassay and interassay coefficient of variation in determining the relative levels of expression? A semi-quantitative analysis based on the ratio between densitometric values probably results in a rather high degree of variation and any strong conclusive statement should be avoided.

Fig. 6 and 7 do not add any important information and should be omitted. Conversely, the densitometric values (mean + SE), in addition to the “p” values, should be detailed in the text.

In summary, the quality of the manuscript was improved by further experimental work, which allowed a semi-quantitative analysis of the results. However, the authors should only comment that GATA-4 expression appears to correlate, yet in a limited series of adrenal tumors, with a more aggressive behavior. Any other statement appears to be only speculative and not well supported by the present results. The discussion should be modified accordingly (i.e. page 13, last paragraph).