The authors have a publication history of anticancer drug tissue penetration, whereas Dr. Tannock has a long history of research in clinical trials, quality-of-life analyses and the interaction between the tumor microenvironment and chemotherapy.

The authors now present data on immunohistochemistry to quantify the distribution of the monoclonal antibodies cetuximab and trastuzumab in relation to blood vessels and to regions of hypoxia in human tumor xenografts.

The manuscript is well written and generally adheres to a straightforward explanation of the conducted experiments.

There are however several major issues that need clarification and a very severe general problem. I will address some of these issues now in detail:

An issue that should be addressed is the determination of the selected antibody doses for mice. There is no explanation within the manuscript how the dose for cetuximab or trastuzumab was determined. Of course there is a large body of information on these two antibodies and both are used in clinical routine. The decision on the dosing however should be explained.

On page 9 the authors conclude "These data confirm relatively uniform distribution at 24-48h after injection of the higher dose of 1 mg cetuximab" for mice bearing A431 xenografts. Looking at table 1 I found some inconsistencies that were not addressed and question the above statement. First, there is a SEM of 6.9 for a dose of 0.01 mg of cetuximab at 100µm and 4.3 for the 1.0 mg (each after 24h). Given the statistical basis of these observations, it is not sound to use these values for concluding a uniform distribution (or in the case of 0.01 mg a "non-uniform" distribution). Another (minor) aspect is the technical variability using fluorescent staining and microscopy, which implies more questions as to the observed variations.

The here presented data do not allow to draw a clear conclusion. It is even more unsettling to see similar staining intensities between 0.01 and 1.0 mg. Another aspect is the high staining intensity with 0.05 mg after 24h at 20µm, an intensity that is higher then the intensity with 1.0 mg at 24h at 20µm. How can this be explained? Is there a in-between-mice variability? The authors give no explanation. There is another problem with the tumor tissue. On figures 3 and 4 there are three sections of tumor tissue depicted. However the bottom images
(C) show no visible hypoxic areas (green). Even if there are some hypoxic (green) areas the question is: how can it be that the architecture is so different? Is the tumor changing its histomorphological aspects within the hours between 4 and 48 hours? Or is the variability between animals so strong with respect to the tumor morphology? How is the distance to hypoxia measured, if no hypoxic regions are visible (Figure 3 C)? Would one see the distribution of monoclonal antibodies (blue) in hypoxic regions (green) as overlay?

The authors do not address these severe problems. If the tumor heterogeneity is so strong, then the authors should explain how they could achieve a reproducible measurement.

On page 12 the authors discuss that „minimal drug distribution to hypoxic tumor cells“ might be due to less receptor expression. This could easily be addressed experimentally by a receptor counterstain with a different (e.g. polyclonal) antibody to determine expression levels as well as binding specificity in the tissue, especially in hypoxic regions.

On page 12 the authors write "At short intervals after injection of all doses there is a concentration gradient of staining intensity of the antibodies with increasing distance from blood vessels within tumors that strongly express the target receptor." They clearly overinterpret the trastuzumab data they present in figures 4 and 5. So these points should be clarified by discussing in more detail the observed differences, putting them in a statistical context and also addressing the above mentioned intensities of cetuximab at 0.05 and 1.0 mg.

Another problem is the observation: "At moderate and high doses the distribution then becomes more uniform with time [...]". It was already published (Blumenthal et al.) that an irrelevant antibody was found to be homogeneously distributed 3 days after injection, even at a low protein dose. This should at least be discussed in the context of the actual data.

A severe general problem with the present manuscript lies within the scientific aim and key message. The authors conclude that there is a limited (resp. heterogeneous) distribution within human tumors. Poor drug binding in hypoxic regions was observed.

These findings were already published in many different studies (Peter L. Jones et al. 1986, Martijn G. Steffens et al. 1999, Gregory P. Adams et al. 2001, Tsuneo Saga et al. 1995, Mark S. Dennis et al. 2007, Greg M. Thurber et al. 2008), especially pointing out the perivascular aggregation and the heterogeneity within the tumor.

The current manuscript therefore has little novelty and I wonder, whether the authors wanted to point out a different aspect in their work.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests