Reviewer's report

Title: Quantum dots in sentinel lymph node mapping: biodistribution study

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Reviewer: Sanjiv Gambhir

Reviewer's report:

General

The article needs major editorial revisions for the English language. It was distracting and sometimes difficult to understand some of the basic objectives. Under different circumstances it may have kept a reviewer from even attempting to read the article. Overall, the experiments seemed simple enough. The authors describe a method by which they track the distribution of Q-dots (15-20 nm) after injecting them into the paw (intradermally). It is confusing whether or not they used a tumor model to track q-dots to the sentinel node, or whether the "sentinel node" is just the immediate set of lymph nodes encountered after intradermal injection into the paw.

I appreciated the detail the authors gave on specific concentrations of the Q-dots they used in the methods section. Since this was a biodistribution study, there were no negative results (i.e: they just reported what they observed), and most of their work correlated well with what others had reported in the literature. Since this study used no targeting mechanism, the control they used (PBS) seemed sufficient. Not surprisingly, they found that more fluorescent signal came from Q-dots rather than PBS.

They also reported that Q-dots (15-20 nm) get to the sentinel lymph nodes as early as 5 minutes post paw injection, and remain there for at least 24 hrs. Although not identical, a similar paper that was NOT referenced by these authors (Ballou et al 2007, Bioconj. Chem.) reported early detection of q-dots (22-41 nm) (similar concentrations as this paper) traveling toward inguinal nodes as early as 3 minutes after intra-tumoral injection. The authors fail to cite this important reference! They were also able to monitor the q-dot distribution out to 24 hrs. However this paper in review seems to take a more quantitative look at the biodistribution by comparing it to the amount injected and looking at other organs as well.

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

1. The paper must be grammatically edited properly before considered for publication.

2. Did the authors use a cancer model? I can't find a cancer model used in the paper. If not, why didn't the authors use a cancer model? Cancer that has
metastasized to the associating lymph nodes may cause blockage throughout the lymphatics and will have a different biodistribution pattern in non-tumor bearing mice. It should be stated in the title or abstract that this study tracks the distribution of Q-dots within normal nude mice. Why is it termed the sentinel node if there is no tumor from which drains to it? Consider changing the wording to regional draining lymph nodes instead of sentinel lymph nodes which are more commonly defined as the hypothetical first group of nodes reached by metastasizing cancer cells from a tumor.

3. Another concern is that they were unable to get tighter error bars in Figure 4. Especially when this is to be ultimately used clinically. It was also unfortunate that they were unable to detect a difference between PBS and Q-dots without having to make and incision in the mouse. This is NOT a non-Invasive technique as stated in the paper. It seems that this technique has it's advantages in being able to sensitively detect as little as 20 pmol of Q-dots within 5 minutes after injection. However the limitations may outweigh this perk in not being able to detect Q-dots without making several incisions through the skin (not clinically friendly).

4. From a personal prospective, I feel that the authors need to be more convincing in explaining why this method would be preferred over the existing lymphoscitigraphy method with radioactive 99mTc. Their proposed method with Q-dots has serious depth of penetration limitations unlike radioactive 99mTc, and the radiation dose that one receives for this study is well understood and not deemed "dangerous". However the unknown toxicity effects of the Q-dots remains to be seen, especially if the majority of the Q-dots are staying near the injection site as stated in this paper. Overall the reasoning to replace the current clinical methods with this Q-dot technique is not convincing. The authors reasoning is that radioactive material is "unsafe" and that blue dye can cause anaphylactic shock and unflattering blue staining, but the advantages they list for Q-dots (resistance to photobleaching, high quantum yield and cross section) are advantages over other optical probes, not radiation and blue dye. It needs to be worded better. It is still unknown how toxic Q-dots will be in humans, you can't claim that radiation and blue dye are "unsafe" (yet they are used in the clinic) and suggest that q-dots are, even though it is still unknown.

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. Aside from the grammar in the text, Table 2 is mislabeled where the numbers have commas (,) instead of decimal points(.).

Discretionary Revisions (which the author can choose to ignore)

2. What about other lymph nodes in the body, were they taken for q-dot evaluation? It would be interesting to see how far the Q-dots could travel through the lymphatics. Can Q-dots be labeled with blue dye? It's always good to have an extra way of validating Q-dot biodistribution.

3. Again, the author needs to more concisely organize their thoughts on how this
method will be advantageous over the current lymphoscintigraphy method. There are several news articles that describe it better in laymen’s terms.

4. The author’s don’t discuss much about the fluorescence excitation laser fiber optic probe. Is the energy high on this laser where it will destroy surrounding tissue?

**What next?:** Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

**Quality of written English:** Not suitable for publication unless extensively edited

**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