CRACK IT DRGNET – Questions and Answers

Q: Will it be possible to match the phenotype of DRG neurones freshly harvested from humans with that of healthy neurones derived from stem cells?

A: We do not see this as an issue, but rather a good opportunity to better understand and characterise the phenotype of harvested primary human DRG neurones and to discern their limitations. It would be interesting to compare the phenotype of these cells with that of stem cell-derived neurones.

Q: Have you experienced any problems with cryopreservation and reconstitution of primary DRG neurones?

A: We have shown that both primary whole rat DRG neurones and dissociated material remain viable after being frozen and reconstituted, as determined by action potential generation, the expression of functional sodium, calcium and potassium channels, and appropriate response to challenge with well characterised agents such as capsaicin.

We don't envisage this being a problem for human primary DRG neurones.

Q: Are you interested in DRG neurones derived from stem cells?

A: Our primary interest is in freshly isolated primary human DRG neurones and this is the focus of the Challenge. We would be interested in stem-cell derived DRG neurones from the point of scalability and in the development of iPS cells from patients more susceptible to pain as interesting disease models. However we need the primary human DRG neurones to be able to assess how representative the stem cell-derived DRG neurones are.

Q: Do you envisage the DRG neurones coming from a single or multiple sites?

A: To be able to deliver a regular supply of cells to the research community (in the order of millions of cells per week) we anticipate the establishment of a network of multiple sites. However it is up to the applicant to make the case if they think they can meet the Challenge deliverables with a single site.

Q: How do you get past the heterogeneity differences in populations of cells?

A: It may be possible to address this by examining patient to patient heterogeneity or rostral to caudal heterogeneity. This may be something the sponsors can offer to do as an in-kind contribution. The heterogeneity of sensory neurones is itself an interesting question, and one that could only be fully addressed by availability of viable sensory neurones suitable for physiological and pharmacological testing.

Q: Is it possible to conduct the heterogeneity experiments in post-mortem tissue?

A: Post mortem tissue is clearly a useful resource for expression studies and transcript expression. Neuronal activity is dependent on the combined activity of multiple ion channels, and expression studies and transcript profiling do not address the issue of the role of individual factors in neuronal activity. Post-mortem tissue is not of sufficient quality to enable physiological testing; availability of physiologically relevant material would allow functional characterisation of neural heterogeneity and increased understanding of the role individual channels play in neural activity, and would complement and extend experiments in post-mortem tissues.

Q: Is there a difference in the response of primary human and rat DRG neurones?

A: There has not been sufficient physiological characterisation of the human DRG system to be able to answer this question.

Q: Would you prefer cell collection to be conducted in the UK or from across Europe?

A: We have no preference as long as a regular supply of good quality neurones can be provided.

Q: Do you envisage there being a problem gaining approval from ethical committees because of the industry partners on this project?

A: The use of the DRG neurones by the industry partners will be purely for basic research purposes. It is true that the improved knowledge gained from these studies may enable the development of new, marketable drugs, but this is also true of the academic partners. We don't see this as being an issue for this project.

Q: Are you interested in more complex cell culture approaches, e.g. using cell guides, etc, once you have the DRG neurones?

A: Not at this stage of the process. We are more interested in ensuring the cells have been harvested correctly and characterized fully and that it is possible to provide a regular supply of these cells for research purposes.

As the programme of work develops it may become appropriate that the development and provision of tools to facilitate cell culture for assay purposes be considered. The sponsors do not expect to see these as an integral part of any application and if included it must not be at the expense of the more important DRG supply and characterization work.

Contact the NC3Rs if you have further questions about this challenge and we can facilitate communication with the Sponsor.

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