5th Meeting for the MITA Validation Study

January 30 & 31, 2020

VMT: E. Corsini, D. Germolec, T. Inoue, S. Aiba, Y. Kimura, T. Omori, N. Mashimo, K. Okayama, H. Kojima, T. Ashikaga, S. Venti

Participating Labs: R. Yasuno, Y. Nakajima

	January 30
Kojima:	Today is the 5th meeting of the MITA VMT. (See agenda.)
Omori:	(See presentation.) Although we judge each chemical to be either positive or negative, I think it is important to emphasize the dose response information shown on the graphs.
Aiba:	It is interesting to see chemicals like No. 13 show borderline results.
Germolec:	There are some graphs that are visually similar but have different results. And this is a little bit strange.
Omori:	The results are determined by an algorithm, which does not "look" at the graph.
Aiba:	But the between-lab reproducibility is 80%. We have to wonder if the criteria are entirely correct, but we cannot change them now.
Kojima:	For the validation study, we have to classify each chemical as positive or negative, but in the future, we need to consider the needs of users who are classifying new compounds.
Corsini:	Without knowing what the chemicals are, we cannot consider any biological factors. For now, we must just base our classifications on the numbers. But there will always be some false positives or false negatives when the results are close to the threshold value.

Germolec:	But the fact that the between-lab reproducibility is very good is a very positive thing, irrespective of these borderline results.
Inoue:	What about the I.I.-SLR-LA valve of ≥ 0.05?
Aiba:	We have several ways to determine cell viability. This assay measures the transcription of housekeeping genes. And the cells maintain their membrane integrity. So, this number is analogous to 80% cell viability.
Kojima:	The data sheets are available on the JaCVAM website. (See username and password shown separately.) If you agree that the data is acceptable, we can open the code sheet.
All:	We achieved our goals for within- and between laboratory reproducibility, and we agree that the data is acceptable.
Aiba:	The Luster data is difficult to use for reference data. So, we looked through the literature, and compiled this list. I would like to add more but I am not sure what is available. But there are many chemicals that we do not have data for. So, this is a problem we must solve.
Corsini:	Without reference data, we cannot define predictive capacity, but what we can try to do is use the clustering to see how many in vivo positives we missed. It will never be a standalone test, so we should use the cluster data to see how it correlates. And there are some chemicals that have discordant results.
Germolec:	There are some chemicals we might have data for, but it would difficult to create a list of chemicals we think are positive in vivo. But this test is not going to be standalone, it will be just one tool in the toolbox.
Corsini:	We should stay withing the MITA, because there are also differences in human response for macrophage and monocytes for IL-1 A or B. This study shows good reproducibility, so this assay should be evaluated only within the context of MITA. Because this assay is testing only a very small part of the immune system at large. What is important is to evaluate the predictive

	capacity of MITA as a whole, not just this individual test. Of course, we will need to explain this.
Germolec:	We have looked at predictive capacity of the IL-2, so how does that improve if we add the IL-1 assay to that?
Aiba:	Unfortunately, the IL-2 assay covers most of the results from the IL-1 assay.
Inoue:	Perhaps changing the main components will reveal the third axis more clearly.
Corsini:	Perhaps at the end of the validation we can see how they fit with the cluster data and if IL-1 adds to predictive capacity.
Germolec:	Possibly, the addition of the IL-1 assay will not increase predictivity. It is possible that the IL-1 will not be needed for all permutations of the modified MITA.
	(Lunch break)
Germolec:	I will provide Dr. Aiba a list of chemicals that can be used for reference. So, it seems that we have a way forward to say something about predictive capacity. But even if we cannot assess the predictive capacity because of a lack of in vivo data, that does not imply a limitation of the assay.
Kojima:	The OECD is now promoting the development of AOPs
Germolec:	I think in terms of immunotoxicity, we are going to have to show how multiple AOPs intersect.
Aiba:	The immune system is highly redundant, so there are many cytokines that need to be described. But that does not mean that each of these cytokines presents a specific effect.
Corsini:	There are a lot of chemicals that have been tested with the whole blood assay, so it might be interesting to compare with those results.
Germolec:	Do we need to go over what our follow up activities are for predictive capacity?

Inoue:	Does the IL-1 data ignores augmentation, but the IL-8 Luc assay includes augmentation? So maybe the vector will be different?
Aiba:	Yes, that is correct.
Kojima:	If we have not proposed it to the OECD, we will need to complete the validation study and peer review report next year.
Inoue:	In the table with information on monocytes, is this data from human monocytes? It would be good to know which is human and which is animal monocytes.
Aiba:	Yes, it shows whether it is human or animal.
Corsini:	Maybe we could add a column to show positive or negative results for NTP to this table.
Aiba:	We will have to decide that ourselves from the data we got from Dori.
Germolec:	I'm not sure we have data for all the chemicals that Dr. Aiba has.

	January 31
Kojima:	Today I would like to discuss the validation report and related issues.
Corsini:	Item 4-3 should not be T-cells. This is a monocyte cell line.
Aiba:	(Review of who will revise each section of the ToC.)
Germolec:	As we discussed yesterday, we need to be careful with the predictivity. We might want to remove parts of section 10 on predictivity for now but discuss in section 10-7 about potential of the assay within MITA. This is a strong assay, but we don't have an appropriate data set with which to discuss predictivity as a standalone test.
Omori:	Also delete section 8-3-6 from my section.

Corsini:	Also introduce this issue in section 2 about the object of the study. The majority of chemicals are well-known immunotoxicants for which in vivo data is available, so we have lots of data that is relevant to judging whether or not MITA is a suitable model for predicting immunotoxicity.
Aiba:	Once I revise the discussion, I will share with the VMT for comments.
Omori:	I can discuss clustering but after that it is not possible to discuss predictivity without a data set for comparison.
Aiba:	We might be able to identify and characterize several different clusters. I will make IL-2 assay the first tier, and then IL-1 or IL-8 as the second tier. And if we have a positive result in the first tier, then we accept the positive result.
Corsini:	We need to be clear about what we consider immunotoxicity and what we consider skin sensitization when comparing in vitro with in vivo results.
Germolec:	The IL-8 with give you a yes or no for skin sensitization, but you are still going to need another tier for immunosuppression. So, it might be interesting to look at the predictivity of different combinations of the IL-1, IL-2, and IL-8. You might find that you only need one or two rather than all three tests.
Corsini:	The IL-8 Luc is similar to in vivo testing in that it challenges the immune system.
Germolec:	I will try to identify some chemicals that target monocytes and macrophages from the Tox21 data.
Corsini:	An added value of MITA is that it gives some additional information about whether the chemical acts on acquired immunity or innate immunity.
Aiba:	The IL-8 Luc in the presence of LPS could add some predictivity.
Kojima:	My concern is the use of different solvents. DMSO and EXVIVO will result in different predictive capacities.

Corsini:	Maybe we can note that the performance is very good and that we are responding to the need to remove serum from the system by using EXVIVO culture medium.
Kojima:	But I think the solvent will affect the clustering analysis.
Aiba:	In this context, I don't think the use of EXVIVO really matters. I wonder if I need define an applicability domain when trying different combinations.
Corsini:	When you study predicative capacity, you will need to consider an applicability domain.
Germolec:	You can explain what you think the applicability domain should be in the discussion.
Corsini:	In places where we have a discrepancy, the applicability domain provides additional information that helps us understand why. After you determine your tiered approach, maybe we can have a conference call to discuss the results. We are more concerned about missing a positive than about overpredicting a negative as positive.
Aiba:	Dori, when can you get us the information about chemicals?
Germolec:	I would say end of March. But I also need to see what action items I am responsible for before I can promise. One thing is that the calls—positive or negative—I can get by the end of March, but it will probably take until the end of summer for the chemicals on which you need more detailed information.
Aiba:	I think there will be 19 chemicals for which I would like detailed information.
Germolec:	So please send me that list.
Kojima:	When do we think we can finalize the validation report?

Aiba:	By the end of September?
Kojima:	So, we will need to have conference calls in October or November.
Omori:	Will you do more testing?
Aiba:	We have to retest some chemicals so they can be judged on new criteria. And there are some that have never been tested using IL-8 in the presence of LPS. So, I might have to request some additional testing from the participating laboratories.
	Action Items ✓ Dr. Germolec will send Dr. Aiba a file will all Tox21 chemicals that were tested for immunotoxicity by the end of February. ✓ Dr. Aiba will send Dr. Germolec a list of 19 chemicals for which a literature search and detailed information is requested. Dr. Germolc will respond by the end of July. ✓ Dr. Kojima will coordinate a conference call for May or June. ✓ Dr. Aiba will revise the validation report by the end of September. ✓ Dr. Kojima will organize a final F2F meeting to be held in November.