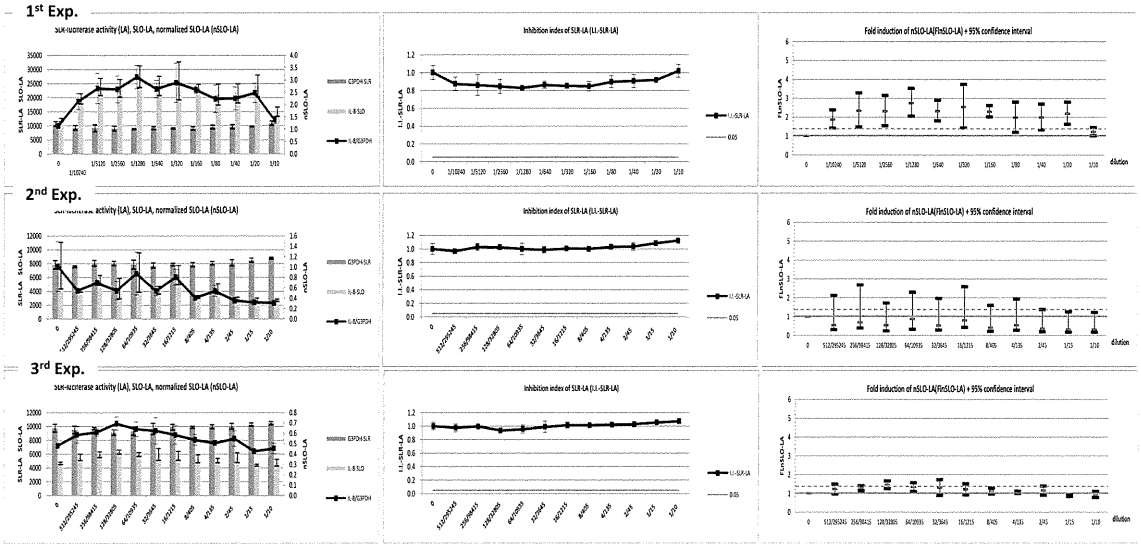
〈Lithium carbonate〉



厚生労働科学研究費補助金（化学リスク研究事業）
免疫毒性評価試験法Multi-ImmunoTox assayの国際validationへ向けての検討
分担研究報告書

国際バリデーションの施行

分担研究者　小島　肇
国立医薬品食品衛生研究所

研究要旨

新たな*in vitro*免疫毒性評価試験法（Multi-ImmunoTox assay：MITA)のOECD（Organisation for Economic Co-operation and Development）試験法ガイドラインとしての公定化を目指し、国際バリデーションに向けた活動を行った。国際的な専門家を招聘して本試験法に対して意見を求めた結果、平成28年度以降、MITAに関する国際バリデーションを開始することになった。

キーワード：免疫毒性、動物実験代替法、バリデーション

A．研究目的

新たに開発された *in vitro* 免疫毒性評価試験法（Multi-ImmunoTox assay：MITA）の OECD（Organisation for Economic Co-operation and Development）における試験法ガイドライン（Test Guideline：TG）を目指し、国際バリデーションの開始を模索する。

B．研究方法

B-1. 国際的な専門家との意見交換

平成28年度以降、MITAに関する国際バリデーションを開始し、国際的なTGへの道程を明確にすることを予定している。その第一歩として、平成28年１月国際バリデーションのキックオフ会議を企画した。

B-2.バリデーション研究の被験物質選択

キックオフ会議において、Phase0として、トレーニングに用いる被験物質およびphase I として施設内再現性を評価するための被験物質の選択を行った。

B-3.IL-8 Lucアッセイの公定化

平成27年10月、OECDの皮膚感作性試験専門家会議に参加し、日本で開発されたin vitro皮膚感作性試験のうち、IL-8 LucアッセイのTGとしての意義、必要性について意見交換した。

C．結果

C-1.　国際的な専門家との意見交換

国際バリデーションのキックオフ会議には、免疫毒性およびその試験法に関する専門家として、海外から Dr. Emanual Corsini (Milan Univ.)、Dr. Erwin L. Roggen（3Rs Management and Consulting ApS）および Dr. Dori Germolec（NTP/NIEHS：電話でのみ参加）を、国内からは、景山茂樹博士（富士フィルム）および日本免疫毒性学会の推薦者である井上智彰博士（中外製薬）を外部専門家とし

て招聘し、研究班の班員を含む表１に示すメンバーにて２日間掛けて、MITA の科学的意義、試験法プロトコルの妥当性などについて討論した。会議の議事次第を添付文書１に示す。

会議に先立ち、研究代表者の相場は、当初の計画を一部変更する以下の提案を示した。

１）細胞の選択と測定指標の妥当性

平成 28 年度からの 2 年間は IL-2 レポーター細胞の 2H4 を用いてバリデーション研究を実施する。この細胞は今年度に作成した 60 種類の化学物質からなるデータセットにおいても鋭敏に IL-2 転写活性抑制物質を検出することができている。したがって今年度に作成した IL-2 転写抑制を指標としたT細胞サブセット分化異常の AOP に則った評価も可能である。この IL-2 の結果とすでにバリデーション研究が終了している IL-8　Luc アッセイの組み合わせにより、免疫毒性を評価することとした。

２）一つ一つの試験法を独立してバリデーション研究を行うべきであるが、そのスケジュール

これから行うバリデーション研究においては予算の関係もあり、上記 IL-2 転写活性抑制に関する試験法に関してのみ施行する。

３）アンタゴニスト試験系に対する懸念（これまでの経験ではアンタゴニストのプロトコルがよく練られていないと頓挫する可能性が高い）

Lipopolysaccharide (LPS)に対するアンタゴニストを用いる評価系は、現時点では再現性が不十分である。現在、種々検討しているところである。

４）試験法データを組み合わせた判別式の確立

今後、作成する予定である。

この案をもとに表１に示すメンバーにて議論を重ねた。その議事録を添付文書2に示す。主な論点を以下に示す。

・免疫毒性は多岐に渡り、IL-2やIL-8にエンドポイントを特定することが妥当か。
・IL-2は免疫毒性に重要であり、事実的なエンドポイントである。作用機構の点から有用である。
・細胞毒性は免疫抑制の作用機構の一つであり、IL-2を細胞毒性の代用として測定すべきである。
・IL-2が関与したAOP(Adverse Outcome Pathway)の開発も必要である。
・評価には他の試験結果との組み合わせが必要である。
・バリデーション研究の開始は早すぎる。バリデーション研究を行う理由が見当たらない。
・偽陰性の原因を明確にすべきほうが重要である。
・今すべきことは、ヒトや動物の免疫毒性データを整備することである。
・MITAはスクリーニングツールとして有用であり、真の陰性結果を見つけられることを確かめるためにバリデーション研究は必要である。
・IL-2レポーター細胞のもとであるJarkat細胞は他の細胞よりは再現性が得られやすい。
・バリデーション研究を実施するなら、再現性の確認のために実施すべきである。80%の施設内再現性が必要である。
・被験物質の選択が重要である。
・数年前に作成した免疫毒性物質リストと比較して、相場らの選択物質は主な免疫毒性物質を網羅している。
・過去の経験から、陰性物質を見つけることが難しい。
・まず明らかな陽性、陰性物質を含む5物質で結果の一致性を確認すべきである。
・プロトコルに細胞の管理や最大適用濃度を明記することが重要である。

以上の議論の末、再現性を確認するための国際バリデーション研究の実施に合意が得られた。

早速、添付文書3に示すバリデーション計画案を示し、外部専門家の意見をもとに、平成28年度以降に実施するバリデーション計画を検討した。まずphase0としてトレーニングを実施すること、次に

phase1として施設内再現性を確認する計画に合意を得た。

C-2.バリデーション研究の被験物質選択

まずはphase 0 として、3施設のトレーニングを行う5物質を選定した。表2にそのリストを示す。

施設内再現性を確認するphase I の5物質も選定したが、コード化して実施することもあり、本報告書には記載していない。

C-3.IL-8 Luc アッセイの公定化

OECD における専門家会議において、日本からOECD に提案している皮膚感作性試験代替法 IL-8 Luc アッセイが専門家会議で議論された。議事概要を添付文書 4 に示す。

日本から開発者の相場らが本会議に参加し、概要説明を行うとともに、内容の詳細について議論した。まだ第三者評価が進行中であるが、TG 化が期待される IL-8 Luc アッセイの国際的な理解が深まった。

D. 考察

免疫毒性は多岐に渡り、作用機構がわかっているものは少ない。そのような状況下でIL-2やIL-8に特化した試験法の開発には懐疑的な意見が外部専門家からあった。

とはいえ、IL-2が免疫毒性の重要なエンドポイントであることは間違いなく、この試験法の開発を中断するほどの大きな理由は見当たらない。そこで、再現性の確認を目的としたバリデーション研究を行うことで外部専門家の合意が得られた。その際の被験物質の選択も重要であり、phase 0を経て、phase Iの5物質で施設内再現性を確認した後、施設間再現性を確認するためのPhase IIの被験物質選択が重要との見解で一致した。

E. 結論

新たな*in vitro*免疫毒性評価試験法（MITA）のOECDにおける公定化の道筋を明確にするため、国際的な専門家を招聘して意見を求めた。

その結果、平成28年度以降、MITAに関する国際バリデーション研究を開始することになった。

F. 添付文書

1) Agenda：Kick-off meeting for the MITA assay
2) Minutes of MITA Kick-off meeting, Jan. 27 & 28, 2016
3) Study plan for the validation trial on multicolor reporter assay using IL-2 Luc (IL-2 Luc assay) as a test evaluating the immunotoxic potential of chemicals
4) Draft Summary Record: Expert Group Meeting on Skin Sensitisation, 14-15 October 2015, Paris, France

表１．MITA第一回国際バリデーション会議　参加者リスト

No.	Name	Affiliation	Country
1	Emanuela Corsini	Universit.AN` degli Studi di Milano	Italy
2	Erwin L. Roggen	3Rs Management and Consulting ApS	Denmark
3	Shigeki Kageyama	Fujifilm Corporation	Japan
4	Tomoaki Inoue	Chugai Pharmaceutical Co., Ltd.	Japan
5	Setsuya Aiba	Tohoku University Graduate School of Medicine	Japan
6	Yutaka Kimura	Tohoku University Graduate School of Medicine	Japan
7	Yoshihiro Ohmiya	National Institute of Advanced Industrial Science and Technology	Japan
8	Rie Yasuno	National Institute of Advanced Industrial Science and Technology	Japan
9	Kohji Yamakage	Food and Drug Safety Center, Hatano Research Institute	Japan
10	Takashi Omori	Kobe University	Japan
11	Shihori Tanabe	National Institute of Health Sciences	Japan
12	Hajime Kojima	JaCVAM, National Institute of Health Sciences	Japan
13	Steven Venti	Translator	Japan

表２．Phase 0 トレーニング用物質

Chemical	CAS No.	MW	Physical state	MITA IL-2 result
2-Aminoanthracene	613-13-8	193.24	Solid	S(-/-/-)
CH_3HgCl	115-09-3	251.08	Solid	+-
Chloroquine diphosphate salt	50-63-5	515.86	Solid	S(-/-/-)
Citral	5392-40-5	152.23	Liquid	S(+-/+-/+-*)
Dexamethasone	50-02-2	392.46	Solid	S(-/-/-)

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25) 九十九 英恵，松成 夏美，小島 肇，板垣 宏：タンパク質のアレルギー性を評価するin vitro試験法の開発，日本動物実験代替法学会　第28回大会 (2015.12)(横浜)

26) 小島　肇：OECDで検討されてきたin vitroスクリーニング法，環境ホルモン学会　第18回研究発表会 (2015.12)(栃木)

27) Furukawa M, Sakakibara T, Ito K, Sasaki S, Koshita M, Okumura S, Kawamura K, Matsuura M, Kojima H: Histopathological Findings on the Cornea in the Bovine Corneal Opacity and Permeability Test (BCOP Test) for Alternative to Eye Irritation Test, 55th annual meeting of the Society of Toxicology (2016.3) (New Orleans, U.S.A.)

28) Narita K, Vo P.T, Nakagawa F, Kojima H, Itagakai H: Reducing False Negatives of Chemicals in the in vitro Skin Sensitization Test, 55th annual meeting of the Society of Toxicology (2016.3) (New Orleans, U.S.A.)

29) Tsukumo H, Matsunari N, Sugiyama M, Toyoda A, Kojima H, Itagakai H: Development of an in vitro test for Allergenic Potency of Proteins, 55th annual meeting of the Society of Toxicology (2016.3) (New Orleans, U.S.A.)

H. 知的財産権の出願・登録状況

（予定を含む。）

1. 特許取得

なし

2. 実用新案登録

なし

3.その他

なし

Agenda
Kick-off meeting for the MITA assay

Date : January 27, 2016, 13:00 - 17:00
January 28, 2016, 9:00 - 13:00
Venue : New Mitoya, Sendai, Japan (http://www.mitoya-group.co.jp/access/)
Participants: Corsini, E., Rogen, E., Kageyama, S.
Aiba, S., Kimura, Y., Yamakage, K., Ohmiya, Y., Yasuno, R., Omori, T., Kojima, H., Tanabe, S.

January 27
Introduction (13:00-13:10)
1. Welcome address and house keeping (Kojima, H.)

Chair: Ohmiya, Y.

Presentations (13:10-17:00)
2. Development of AOP on immunosuppression in EGMST, OECD (Kojima, H.)
3. Outline of the MITA assay (Aiba, S.)
4. Research on the immunotoxicity (E. Corsini)
5. Applying Toxicogenomics for *In Vitro* Assessment of Immunotoxicants. (E. L Roggen)

Coffee Break

Chair: Yamakage, K.

6. Research on the immunotoxicity (Kageyama, S.)
7. Research on the immunotoxicity (Inoue T.)
8. Research on the immunotoxicity (S. Tanabe)
9. Development of reporter gene assay (Ohmiya, Y.)

January 28
Proposal of validation study (9:00-11:00)

Chair: Omori, T.

10. Outline of the IL-2 Luc assay (Aiba, S.)
11. Protocol (Kimura, Y.)
12. Results of preliminary test by three laboratories (Kimura, Y.)
13. Study plan (Kojima, H.)

Coffee Break

Chair: Dr. Kojima, H.

14. Discussion and suggestion (11:30-12:30)

Closing session (12:30-13:00)
15. Wrap-up on discussion
16. Future plan
17. Any other business
18. Closing remark (Aiba, S.)

MITA Kick-off meeting 添付資料 2

Jan. 27, 2016

Kojima	Opening remarks and review of the agenda, followed by self-introductions Review of new and pending OECD Test Guidelines Increased emphasis on identifying AOP, because of the benefits of using AOP as a framework for development of IATA for skin sensitization testing.
Corsini	Do we need to discuss which aspects immunosuppressive or immunomodulatory aspects are to be tested?
Roggen	Need to keep it simple but also ensure that all essential elements are included.
Aiba	Presentation on Multi-Immuno Tox (MITA) assay (See presentation.)
Corsini	Presentation on Research in Immunotoxicity (See presentation.)
Roggen	Presentation on Applying Toxicogenomics for In Vitro Assessment of Immunotoxicants (See presentation.)
Kageyama	Presentation on Research in Immunotoxicity (See presentation.)
Tanabe	Presentation on Research in Immunotoxicity (See presentation.)

MITA Kick-off meeting, Day Two

Jan. 28, 2016

Kojima	Agenda will change a little. Dr. Aiba will summarize the proposed protocol and then we will discuss. If everyone agrees, we will discuss whether to pursue this approach or not.
Aiba	(Summary of proposed protocol)
Omori	I would like to discuss whether this is a viable test or not. MITA appears to be valuable as an immunotoxicological assay. If you agree, I suggest we discuss a validation study. We have completed a validation for IL=8, and we should validate IL-2.
Roggen	Although not a general test for immunotoxicity, it could be useful for some specific things, which we need to identify.
Aiba	I think this modified MITA could be useful to regulators. It should help identify the characteristics of immunotoxicants.
Germolec	Very hard to hear what is being said.
Inoue	With regard to differentiating T-helper cells, IL-2 is related but so is IL-10 and others, so perhaps the results will differ from in vivo testing.
Aiba	Yes, this is not a complete method. But it could be useful in screening chemicals.
Corsini	I agree that as a screening tool it could be useful. Negative results will require additional testing. But immunotoxicity will require weight of evidence, so combined with other tests it will be useful.
Ohmori	These comments seen to suggest that a validation study is needed. Does anyone disagree?
Aiba	Is it possible apply to OECD for a guideline?
Kojima	Immunosuppression is an important factor in safety assessment of chemicals.
Tanabe	What will the endpoint be?
Aiba	We will need to combine with other cell lines, so we would like to validate for 2H4 cell line alone not just MITA.
Roggen	From ECVAM point of view, I am afraid that reproducibility needs to be improved. And from this data, I'm not sure I understand the limits in terms of applicability domain. Also, it seems there were some false negatives, so it seems there is something we don't yet understand.
Aiba	There are so many different chemicals but there is limited information about their toxicity. Most chemicals lack information about immunotoxicity.
Roggen	Yes, we need tests to identify immunotoxins that are not sensitizers, which is

why it is too early for a validation study. Still need more information about reproducibility and how it will be used for screening, etc.

Corsini Perhaps you can comment more about table on page 24.

Aiba The data shows that IL-2 gives consistent results but is still difficult to get results for THP cells.

Corsini If I understand, Jurkat gives good between-lab reproducibility, but the other cell lines are more difficult. Do you have figures for the 60 chemicals for accuracy, sensitivity, and specificity?

Aiba Looking at page 23, there are chemicals that don't give clear results. For example, acetaminophen. Some of these chemicals have known characteristics, but for many, it is not so clear.

Corsini It is difficult to find a compound that is always negative. So the chemical selection will be crucial and not so easy for this validation.

Aiba There are many chemicals that do not have clear information about immunosuppression.

Germolec We are working to find some true negatives, but it has been a struggle. It is difficult to find a chemical that is active but doesn't have an effect on something. So I wonder if we can find a true negative.

Aiba We thought that we had a good negative control, but when we increased the doses, we found it was an immunosuppressant.
Most of the chemicals that affect the pathways can be detected by IL-2 and with just these two tests in the modified MITA, we can detect the characteristics of most chemicals.

Kageyama Compared with other assays, for example, incubation time is longer and concentrations are higher, so I think that this is important. On page 23 these problems occur when time is short or concentration is low.

Roggen With regard to the Fluorescent cell chip method, which uses mouse cells that would be extensively used. We are focusing more and more on human cells now than 10 years ago. It is difficult to find negative compounds, but cytotoxicity is one mechanism of immunosuppression, so perhaps IL-2 upregulation should be measured in the absence of cytotoxicity.

Aiba We do check to see if viability of our cell line contains luciferase activity stimulated by GADPH promoters.

Inoue On page 23, not just sensitivity or cytotoxicity, there are some substances here that give opposite results between Fluorescent cell chip method and MITA.

Ohmori Is the purpose of the validation study to establish the test method? Perhaps we should discuss whether or not there is a need for this test method.

Aiba If we decide that the method is not sufficiently established, we will have to give up this study. I think I have done almost everything necessary to establish the method and categorize chemicals with it. But the situation surrounding MITA is still immature. Perhaps there is no clear need to screen immunotoxicity and there is not clear target. Pharmaceutical makers have a clear need for skin sensitizer test methods but I don't know if there is a need for screening immunotoxicity.
It is probably just a small step to where a need will be established, but we are not there yet.

Roggen I understand your reasoning, but I also wish that work on immunotoxicity will continue. Unfortunately, I do not see a rationale for proceeding with a validation now. Perhaps we need more human and in vivo data about immunotoxicity.

Corsini I see your point, and I think immunotoxicity should be given much more importance. But we are not studying it enough. I agree that we should find some rationale for this study to proceed.

Germolec It is a constantly moving thing. We have a list of 25 compounds. Mostly environmental chemicals. We are still working on making a larger list, so let

me update you all about the status of that list.

Roggen You said the biggest challenge is finding negative substances.

Germolec It is difficult to run through all the assays and find a substance that is truly negative across all parameters. It is often based on cytotoxicity. We were looking at very wide dose-response curves to try to find cytotoxicity and then refine that immunological responses. It has been hard to find immunotoxicity in the absence of cytotoxicity.

Roggen Do you know the Jerka test using 25 genes to identify immunotoxicants? Maybe you need to look at the gene level to achieve that.

Aiba Can we reach a conclusion? The situation is difficult, I know. But I would like to submit an AOP for airway hyper sensitivity or contaminated water. But it is difficult to select chemicals. I would be happy to hear ideas. There is little info on chemicals, key events, and outcome. What chemicals might be useful?

Corsini There are probably close to 100 compounds that are immunotoxic or non-immunotoxic. There was work in the 90s listing non-immunotoxic chemicals. How many do we need?

Germolec There is crossover between the list we are making and the MITA list.

Roggen And to validate, you will need chemicals that affect IL-2 expression.

Corsini Or chemical that affect T-cell expression. You have tested 60 chemicals. How did you select them?

Ohmori One purpose of validation study is to validate reliability. How about that? Most of this discussion is about relevance.

Roggen I think this is a pragmatic approach, since we know IL-2 is important. But to validate that for regulatory use, we have to identify the chemicals that follow that mechanism, and we might not have enough to do that. From a mechanistic point of view, this is an important approach. Reliability is a technical aspect that has to be done but can be improved.

Aiba How can we examine chemicals for immunotoxicity? Is there a method that can be applied to the compounds? It would require a high concentration. How do people examine the effects of pesticides? Skin sensitizers can be tested in a high concentration, but working with low concentrations makes it hard to discern immunotoxicity.

Corsini Pesticides are immunotoxic in animal experiments. So you compare in vivo and in vitro data. Most pesticides today are not immunotoxic, but some old ones are.

Kageyama On page 23, discrepancies are important. By evaluating cytotoxicity, you can examine the effect on T-cells. So combining IL-2 and cytotoxicity is important. So page 23 shows a mixture of two assays.

Roggen All different aspects are important. Here we have two assays that can be combined eventually with other assays.

Ohmori I think this test method is not yet established. We should discuss whether or not this method is valuable enough to establish.

Aiba I think the method is established. We can still modify the assay. We have to optimize certain aspects, but the issue here is that we cannot find a good positive control. But we have no information about a chemical that is clearly immunotoxic.

Roggen I think we agree the tests are important and address specific mechanistic events that are related to immunosuppression. So the test is valuable. The next step is a project to improve reproducibility. Then establish a list of reliable positive and negative controls. It won't be easy but you can do it. And after you have that, you can think about a validation. Chemical selection is crucial to validation planning, and will take time. These are the three steps needed to proceed with this project.

Corsini I agree with Erwin.

Kojima I also agree. It is also necessary to establish AOP.

Roggen	AOPs are never finished, so do that in parallel with other activities.
Aiba	It is a chicken and egg issue. The modified MITA can provide some insight into the characteristics of chemicals. This method will be useful to people who want to identify the mechanism of immunotoxicity.
Ohmori	So, the conclusion is to continue the project. So what is the next step? Should we discuss the plan?
Corsini	It is important to come up with a good list of chemicals that we are confident of in terms of immunotoxicity.
Germolec	Yes we are willing to share what we have and what we will find in the future.
Corsini	We started a few years ago with making a list.
Germolec	Yes, there have been many stops and starts. But we are willing to share.
	Break
Aiba	(Explanation of protocol)
Corsini	PMA has a shelf life. Is there is shelf life to the stock solution? From my own experience, PMA stops working after 6 months, so please indicate a shelf life. Will you include a positive and negative control?
Aiba	See page 23.
Corsini	What is the cost of this cell line?
Aiba	Will be determined in the future.
Roggen	I have a comment about the cells. Cell lines lose functionality, especially in transfected parts. It is important to define the No. of permissible passages.
Aiba	We are now confirming that. But I think more than 30 times. This line is already 5 years old but we are still using it.
Roggen	I understand but you need to define that in the procedure.
Kageyama	What about interference from FCS?
Aiba	We have used this line for more than 5 years, and have changed a couple of times. I don't think it is a factor.
Inoue	On pg 23, DMSO could affect the performance of the cells.
Aiba	Page 27 shows DMSO control
Kojima	Regarding the criteria, the student t-test is perhaps not the best method. Could you change it to a different method?
Aiba	This criteria was accepted for my paper, but if we need to change it for validation, we can do so.
Roggen	From the reviewer's point of view, dose dependent response is important. It would be better to say "and" instead of "or" on pg 36.
Kageyama	Why don't you use beta mercaptoethanol for the T-cell culture?
Ohmiya	Perhaps use a different name rather than 2H4.
Tanabe	How many chemicals per plate?
Aiba	Can test two chemicals on each plate.
Omori	Please send other questions by mail.
Kojima	(Presents draft study plan) Lead lab is responsible for training. Is there a plan for training?
Aiba	Yes, this is similar to earlier assays and we do not anticipate any problem.
Roggen	For within-lab reproducibility, you have 80% acceptance criteria, but I think a rationale for that target is necessary. I always have this question. This figure is very strict. Why is there no target for between-lab transferability?
Kojima	We expect 100% transferability. As to the 80% target, we have five chemicals. So we allow one chemical to be out of concordance.
Roggen	Maybe better to say "4 of 5" instead of "80%."
Kojima	Do you think we need more than 5 chemicals?

Roggen	5 is enough, depending on how clear cut the results are.
Corsini	Is five chemicals enough for within-lab reproducibility with blind chemicals?
Roggen	Five is a common number for within-lab reproducibility.
Kojima	The Phase II will add 20 chemicals, other than the five used for the within-lab reproducibility.
Roggen	The more IL-2 immunotoxicants that you have, the better.
Kojima	(review of schedule) End of Phase 1 study is at the end of this 2016. In the Table 3 it is says Phase 2, but that is a typo. It should be Phase 1.
Aiba	You need 100% concordance?
Kojima	For Phase 0 Study, yes.
Aiba	What happens if there is a non-concordance?
Kojima	Then solve the technical problem.
Roggen	For the five chemicals, the number of positive or negative is not that important. Concordance of results is what is important. (discussion of chemicals)
Aiba	I propose these chemicals for Phase 0: 2-aminoanthracere (DMSO), CH_3HgCl (DMSO), chloroquine (ddw), citral (DMSO), and dexamethansone (water insoluble, DMSO)
Kojima	Is DMSO for positive control?
Aiba	I don't think so.
Roggen	We are looking for five chemicals that are clearly positive? But to challenge the labs, you will want to use chemicals with differing levels of effect.
Aiba	Many of these chemicals are not so toxic, so we can increase the concentration. We have to think about the effect of the chemicals on blood.
Roggen	Is the maximum concentration the concentration that gives the highest toxicity? That is one way of defining it.
Corsini	Max. concentration is 1 mg/mM for Keratinsens and HCLAT.
Roggen	Here we are limited to drugs. We want this test to work for everything. But we need to provide information that will enable people to find the correct concentration for any chemical. There should be some that have only a weak effect, either positive or negative. To see if they can clearly identify it. And then others that are clearly positive or negative.
Aiba	Finding two negatives is a problem.
Corsini	Can we select substances that haven't been tested yet but are known immunotoxicants and affect T-cells.
Roggen	It's better to select chemicals for Phase 1 that have been tested already. For the real validation, you can expand (in Phase 2).
Corsini	So better to select chemicals listed on pages 32 and 33.
Aiba	For Phase 2, I propose: lead acetate ++ (ddw), hydrocortisone + (ddw), dibutyl phthalate +, DMDTC - (DMSO), and nickel sulfate + (ddw)
Roggen	It is good to have one clear positive and one clear negative and then the others that are not so clear. Even if you have to repeat this Phase a couple of times, it is time well spent.
Omori	After checking the properties of these chemicals, Dr. Aiba will discuss the final selection by email. Time to close the meeting now.
Aiba	Thanks for participating. There is still a lot of information that has to be collected and many decisions to make. Thank you for your support.

添付資料３

Version 1,1 January, 2016

Study plan for the validation trial on multicolor reporter assay using IL-2 Luc (IL-2 Luc assay) as a test evaluating the immunotoxic potential of chemicals

IL-2 Luc assay Validation Management Team

INDEX

1. Background

The use of multicolor reporter assay using IL-2 Luc, Jurkat cell (IL-2 assay) is an important for evaluating the immunotoxic potential of chemicals as a part of Multi-ImmunoTox assay (MITA), because of its technical simplicity, short-term test period and accuracy of test result based on a mechanism of immunotoxicity.

The aim of this trial is to (pre)validate the IL-2 Luc assay method to assess transferability and inter-laboratory variability, in order to incorporate this test for screening the immunotoxic chemicals. The IL-2 Luc assay for the validation trial will be undertaken i) in accordance with the principles and criteria documented in the OECD No. 34 Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment [OECD, 2005], ii) according to the Modular Approach to validation [Hartung et al., 2004] ,iii) according to the concept discussed on the validation trials with participation of GLP Test Facilities [Cooper-Hannan et al., 1999] where the whole concept of the validation trials is described in the context of GLP, iv) and in line with the ISO procedure JRC.I.03.GP.01v.01 (http://ihcpnet.jrc.it/quality-safety/quality-documents/unit-03-ivm/doc/JRC.I.03.GP.01v.01.pdf).

The studies part of a validation trial should ideally be performed in accordance with GLP [OECD, 1998-2007; FDA, 1999; EPA, 1998a&b; JSQA, 2010; SCC, 2010]. As a minimum, but not necessary limited, use of standard operating procedures (SOP), adequate data recording, reporting and record keeping are essential.

A general conceptional framework [Hartung et al., 2004; OECD, 2005] will be used for documenting all the study to assess the validation status of a test method, called "modular approach" to validation. In this approach, the information needed to support the validity of the method is organized into modules that provide the following information:

Module 1: Test Definition

Module 2: Within-laboratory repeatability and reproducibility

Module 3: Between-laboratory transferability

Module 4: Between-laboratory reproducibility

Module 5: Predictive capacity

Module 6: Applicability domain

Module 7: Performance standards

The Modular approach as introduced by Hartung et al., allows using datasets from various data sources and studies. This advantage is used in the following proposal to assess the scientific validity of the IL-2 Luc assay. This IL-2 Luc assay for the validation trial has performed under the GLP principle.

2. Objective of the trial

The validation trial will assess the reliability (reproducibility within and between laboratories) and relevance (predictive capacity) of the IL-2 Luc assay with a challenging set of test substances (test items) for which high quality *in vitro and in vivo* data are available.

3. Validation Management Team (VMT)

The VMT encompasses collective expertise with the test, in the underlying science and the scientific design, management and evaluation of a validation trial.

The VMT, which plays a central role overseeing the conduct of the validation trial, includes:

Table 1. Members for IL-2 Luc assay Validation Management Team

Name	Role and expertise	Affiliation
Trial Coordinator Hajime Kojima	VMT trial coordinator , Chemical supplier	JaCVAM, NIHS, Japan (JaCVAM representative)
Lead Lab Yutaka Kimura* Setsuya Aiba*	*Developer of this assay Test method, expertise underlying science	Tohoku Univ., Japan
Shihori Tanabe	Management of quality control	JaCVAM, NIHS, Japan (JaCVAM representative)
Takashi Omori	Data analysis, biostatistics dossier	Kobe Univ., Japan
International expert members		
EU liaison Emanuela Corcini	Test system expertise, validation expertise, immunotoxicity expertise	Milan Univ., Italy
EU liaison Erwin L. Roggen,	Test system expertise, validation expertise, immunotoxicity expertise	3Rs Management and Consulting ApS, Denmark
ICCVAM liaison Dori Germolec	Immunotoxicity expertise	NTP/NIEHS, USA
Japan liaison Shigeki Kageyama	Immunotoxicity expertise	Fujifilm Corporation, Japan
JSIT liason Tomoaki Inoue	Immunotoxicity expertise	Chugai Pharmaceutical Co., Ltd.

3. 1Participating Test Facilities

The laboratories participating in the trial are defined as follow:

Test Facility 1: Hatano Res. Inst., FDSC. Study Director (SD) : Kohji Yamakage

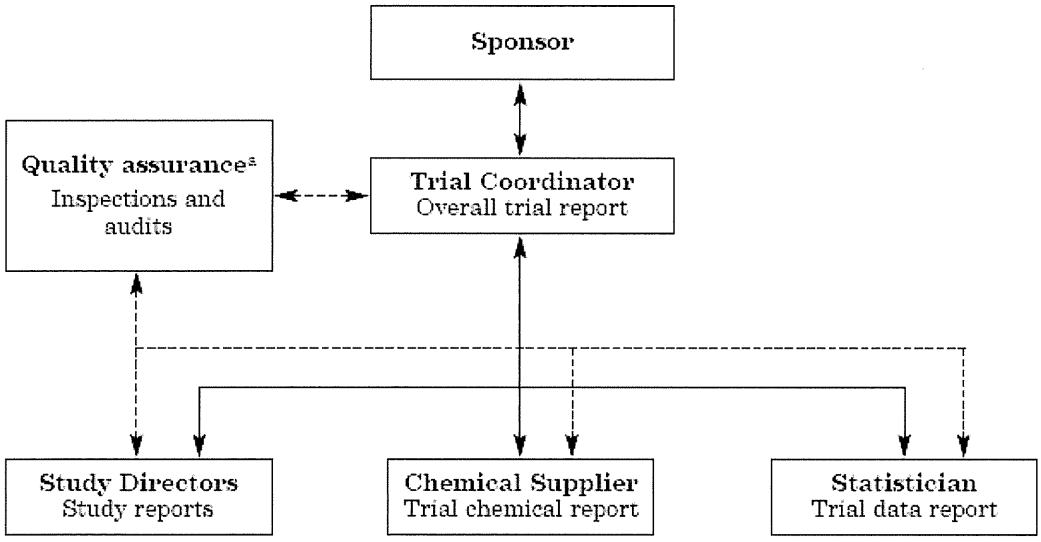
Test Facility 2: AIST, Tsukuba SD : Yoshihiro Ohmiya

Test Facility 3: AIST, Takamatsu SD : Yoshihiro Nakajima

Information relevant for Modules 1, 2, 3 performed by all laboratories. Data obtained by these laboratories have demonstrated that the IL-2 Luc assay is transferable and reproducible between experienced laboratories. The all facility will be the laboratory participating in this validation trial acting as unexperienced laboratory to assess between laboratory transferability, reliability and relevance of the IL-2 Luc assay method under non-GLP conditions (GLP principle).

3. 2 Trial management structure

The management structure of the validation trial is shown in **Figure 1**



[a]*Several Quality Assurance units might be involved in a multi-study trial.*

Dashed lines indicate assurance staff involvement.

Figure 1: Management Structure of the IL-2 Luc assay validation trial

1) Chemical management group

The members of chemical management group are elected by recommendation of the IL-2 Luc assay VMT. They prepare a tentative list of test chemicals and works with the VMT to make a final decision on the test chemicals to be used in the validation trial. The coded test chemicals listed are distributed by JaCVAM.

2) Data analysis group