Good Practice in Traditional Chinese Medicine Research in the Post-genomic Era

GP-TCM

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D5.13

Presentation of findings at the conference planned in work package and discussion with invited experts from the network

Document description	
Name of document	Presentation of findings at the conference planned in work package and discussion with invited experts from the network
Abstract	Summary of WP5's work over the full course of the project including science and technology developments and contributions as well as summary of all work performed i.e. "the WP5 final report".
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TABLE OF CONTENTS

1 INTRODUCTORY REMARKS:

The following report summarizes the main achievements of WP5. These findings have been presented at the GP-TCM final conference **on 13th April 2012.**

Agenda of WP5 Session of the GP-TCM Final Conference

(8.45am-10am, 13th April 2012)

Chair: Dr. Qihe Xu

Minutes: Dr. Fan Qu and Dr. Jue Zhou.

Presentation session (Delivered by: Dr. Xuebin Dong)**:**

1.1 A brief introduction to WP5 objectives: The overall objective of WP5 (Annex I) is to develop collaboration between European and Chinese specialists in biomedical research and/or Chinese herbal Medicines (CHM) in order to:

- give directions for the application of functional genomics to animal studies of CHM;
- optimise research opportunities on CHM through planning coordinated efforts for future directions;
- establish best practice for animal studies of CHM in the EU;
- link together European and Chinese experts in this field.

To achieve our objectives, we designed a 3-year plan divided into 14 deliverables (see below)
Deliverables (brief description and month of delivery)

- D5.1- Kick-off WP meeting and report (**month 6**).
- D5.2- Website building and maintenance (**month 6**).
- D5.3- Building of an expert network in CHM in animal models of disease (**month 6).**
- D5.4- Report on the reviewed literature relating to CHM in animal models of disease (**month 12)**[1]**.**
- D5.5- Circulation and discussion of the report (**month 12).**
- D5.6-Agreed conclusions of the review of literature published (**month 12).**
- D5.7- Elaboration of a priority list of CMH for future research in CMH in animal models (**month 18).**
- D5.8- Report on agreed conclusions on the efficacy of CMH in animal models (**month 18).**
- D5.9- Recommendations on the application of functional genomic studies and chemical synthesis to the study of CHM in animal models of disease (**month 24).**
- D5.10- Recommendations on best practice in CHM in animal models of disease (**month 24).**
- D5.11- Report on the problems carrying out investigations of CMH in animal models (**month 30).**
- D5.12- Future research priority areas in animal studies of CHM (**month 30).**
- D5.13- Presentation of findings at the conference planned in work package and discussion with invited experts from the network (**month 36).**
- D5.14- Final report published in scientific journals (**month 36).**

[1] D5.4 to D5.6 were later merged into a single deliverable: "Review of literature relating to CHM in animal model and elaboration, circulation and discussion of the corresponding report. Report of the agreed conclusions"

1.2 A brief history of WP5 membership and leadership development:

In order to allow members to focus on 1-2 WPs only so that they can contribute with a clear focus, it was decided that around half original WP5 members were lost shortly after the WP5 kick-off meeting.

By the time of the kick-off meeting, the following members were recruited: Prof. Sue Watson (University of Nottingham, UK), Prof. Ping Li (China-Japan Friendship Hospital, China), Prof. Liping Zhao (Shanghai Jiaotong University, China), Prof. Xiaodong Cheng (Shanghai University of Traditional Chinese Medicine, China), Dr. William Weiguo Jia (Shanghai Innovation Research Center of Traditional Medicine, China), Dr. Huige Li (Johannes Gutenberg University, Germany) and Ms. Gemma Olmos Centenera (University of Alcalá, Spain).

In the recent 2 years and half, the following members were recruited: Prof. Y. James Kang (University of Louisville, USA), Dr. Rajendra Kumari (UK, Prof. Sue Watson's local assistant), Prof. Zuguang Ye (China Academy of Chinese Medical Sciences, China), Dr. Xuebin Dong (King's College London, UK), Prof. Peter M. Jones (King's College London, UK), Prof. Shanta Persaud (King's College London, UK), Dr. Ru Yan (University of Macau, China), Dr. Liliana Vargas-Murga (Biothani Europe S.L., Spain), Prof. Jing-Yan Han (Peking University, China).

Professor Bruce Hendry stepped down from the Deputy Coordinator position after the kick-off meeting in 2009; the late Professor Sue Watson took the challenge and led review projects as Deputy Coordinator until she lost her epic battle with cancer in the end of 2011. WP5 dedicates our major paper in the GP-TCM Special Issue of J. Ethnopharmacol. to Sue and the consortium issued a special issue of GP-TCM Newsletter in her honour. At such a crucial time, when the Coordinator Prof. Javier Lucio-Cazaña was promoted to senior management role in his university cannot attend the Final Conference and the Assistant Coordinator Dr. Maria Laura Garcia Bermejo also cannot come to represent WP5 due to a "baby on board" and due shortly after the meeting, Dr. Xuebin Dong took on the challenge and accepted the appointment as the Deputy Coordinator of WP5.

1.3 Year 3 WP5 activities:

- Organised 4 deliverable reports (see item 4).
- Published 1 WP5 paper and 1 WP4/WP5 joint paper in the GP-TCM J. Ethnopharmacol. special issue; one more paper in preparation for publication elsewhere.
- Attended e-MSM and SOP panel teleconferences.
- Presented WP5 research at the 11th Congress of the International Society of Ethnopharmacology Sept 2010, Albacete, SPAIN (3 communications whose abstracts have been published in Revista de Fitoterapia 2010, 10(S1)):
    - ISE3-P40 Animal Models for Cancer Research in Traditional Chinese Medicine *W. Yang , G. Li , L. Garcia, G. Olmos, F. Lucio, X. Cheng*
    - ISE3-P28 Scientific Publications on Animal Studies of Chinese Herbal Medicines (CHM) *Tejedor-N , Garcia-L, Olmos-G , Dong-X, Ye-Z, Kumari-R, Xu-Q, Watson-S, Cheng-X, Li-P, Lucio-F*
    - SE1-P05 Review of Oncology-focussed publications in the field of Chinese Herbal Medicine *Kumari-R, Lucio-F , Garcia-Bermejo L, Watson-S*
- Presented WP5 research at the Traditional Chinese Medicine Symposium July 2011, Braga, Portugal
    - State of the art in animal studies of Chinese Herbal Medicine *Noelia Tejedor, Laura García Bermejo, Rajendra Kumari, Gemma Olmos, Ana Fernandez, Qihe Xu, Sue Watson, Xiaodong Cheng, Francisco J Lucio*
- WP5 webpage and contributed to GP-TCM Newsletters

1.4 Year 3 deliverables:

- D5.11- Report on the problems carrying out investigations of CMH in animal models (**month 30).**
- D5.12- Future research priority areas in animal studies of CHM (**month 30).**
- D5.13- Presentation of findings at the conference planned in work package and discussion with invited experts from the network (**month 36).**
- D5.14- Final report published in scientific journals (**month 36).**

1.5 Year 3 experiences gained and lessons learnt:

There have been no major issues to be reported by WP5 Coordination Team.

1.6 WP5 summary to Year 1-3:

The kick-off meeting **(D5.1)** was held in Alcala de Henares University the days 2 and 3 June, 2009. 11 WP5 members attended the meeting: Xiaodong Cheng, M Laura García-Bermejo, Bruce Hendry, Peter Hylands, William Jia, Rajendra Kumari, Javier de Lucio Cazaña, Gemma Olmos, Susan Watson, Qihe Xu and Liping Zhao. The meeting was highly productive; attendees introduced themselves, their research interests and expertise, discussed the 3-year plan of the WP and assigned jobs related to deliverables for the first year (D5.1 to D5.7). It was agreed that WP5 field work will start with a review of the literature in order: i) to gain an overview of the studies of CHM and, in particular, of the state-of-the-art of the research in CHM in animals and ii) to evaluate the present state of animal studies -and particularly those involving functional genomic approaches- in relation with the need of scientific proof of the efficacy of CHM and the specific problems found in these studies.
In the kick-off meeting was evident that WP5 needed some more experts (we had 6 experts in the beginning) in critical areas. Therefore, we devoted efforts to the building of an expert network in CHM in animal models of disease **(D5.3)**. Accordingly, WP5 has undergone to a major reorganisation which has resulted in a significant increase in experts (now WP5 have 15 experts) covering the critical areas required to complete the WP5 work program.
Our data **(D5.4 to D5.6)** showed that i) research of CHM during the last 10 years had been highly intensified and become more accessible worldwide through increased publications in English, although still most authors had Chinese names (Table 1) and ii) English journals publishing animal research of CHM in the field of cancer (which was chosen as a representative sample of the whole field) were comparable to those publishing animal studies of non-Chinese phytotherapy in terms of impact factor (Table 2).

Table 1: CHM in context

a)

	1950-1999		2000-2011		TOTAL
	ARTICLES	%	ARTICLES	%	
CHM	10732	36.60	18587	63.40	29319
HOMEOPATHY	1978	54.33	1663	45.67	3641
ASPIRIN	22339	64.26	12425	35.74	34764

b)

CHM ARTICLES	PUBLISHED IN ENGLISH		PUBLISHED IN CHINESE		PUBLISHED IN OTHER LANGUAGE	
	ARTICLES	%	ARTICLES	%	ARTICLES	%
1950-1999	4040	28.32	5979	42.58	713	70.52
1999-2011	10225	71.68	8064	57.42	298	29.48
TOTAL	14265	100.00	14043	100.00	1011	100.00

Table 2. Animal studies of CHM in cancer: journals more frequently used in 2000-2009

SOURCE	ARTICLES	%	IF (2008)
ZHONGGUO ZHONG YAO ZA ZHI	40	10.36	------
ZHONG XI YI JIE HE XUE BAO	28	7.25	------
ZHONG YAO CAI	24	6.22	------
WORLD J GASTROENTEROL	23	5.96	2.031
ZHONGGUO ZHONG XI YI JIE HE ZA ZHI	23	5.96	------
J NAT PROD	16	4.15	2.843
AM J CHIN MED	10	2.59	1.058
BIOL PHARM BULL	9	2.33	1.765
CANCER LETT	9	2.33	3.504
J ETHNOPHARMACOL	7	1.81	2.260
J TRADITIONAL CHIN MED	7	1.81	------
LIFE SCI	7	1.81	2.583
ONCOL REP	7	1.81	1.524
CARCINOGENESIS	5	1.30	4.930
ACTA PHARMACOL SIN	4	1.04	1.676
INT J CANCER	4	1.04	4.734

We also found published data on authentication and quality control of CHM, as well as research design of animal studies were far from sufficient to meet the criteria needed to support their reproducibility and reliability. Therefore we proposed the following *checklist to assure a consistent and acceptable quality of herbal research materials:*

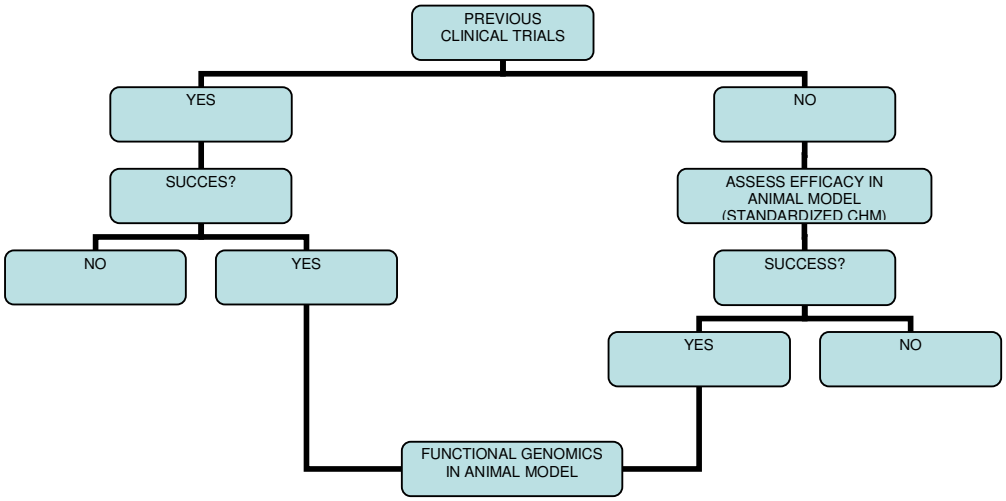
- General records: Harvesting time, parts of the plant collected, regional status, details of any voucher specimen;
- Authentication of the herbal raw material: Taxonomic, macroscopic & microscopic, DNA barcoding;
- Standardization, i.e., to provide quantitative and semiquantitative information about the main active constituents or marker compounds present in the crude drug or herbal products:
  (i) chromatographic and sophisticated modern techniques: spectroscopic evaluation UV–vis spectrophotometry, TLC, HPTLC, HPLC-mass spectrometry, NMR, etc;
  (ii) physical parameters: organoleptic evaluation, viscosity, moisture content, pH, disintegration time, friability, hardness, flowability, sedimentation and ash value.
- Microbiological contamination;
- Pesticide residue;
- Heavy metal analysis;
- Extraction: Solvent used and ratio, time, temperature and yield.
- Tested samples should be kept as voucher samples, voucher numbers should be given and preferably the HPLC proofing should be provided.

After reviewing the existing evidence in the literature of the value of CHM in animal models of cancer and fibrosis (as a sample of the whole field of CHM studies in animals), WP5 team agreed that it is very difficult to generate a priority list because there are not enough good quality studies performed by independent research groups to support efficacy of CHM. WP5 team believes that this conclusion can be extended to the whole field of animal studies of CHM **(D5.7)**. In the case of cancer, there was general evidence of efficacy of the test CHMs in most reported papers-the level of which did vary but the CHMs reported were generally shown to be highly efficacious. However the clinical relevance of these studies was difficult to dissect out and compare to Western medicines as there was little report of toxicity and very little adherence to animal welfare guidelines and ethical committee compliance (only 14% of studies). Therefore some of the effects may have been attributable to non-specific toxicity. In addition there was very few cases where biomarkers of response linked to the mechanism of action of the test CHM were used. Furthermore, experimental design was also compromised as standard of care comparisons were infrequently used (20% of cases). Furthermore the

oncology models on the whole (66% of cases) did not evaluate metastatic spread or clinically-relevant transplantation sites (orthotopic). Also most of the studies were done with established cell lines rather than newly established early-passaged cells from patient tissue (97% of cases). Overall the quality of research in terms of efficacy outputs was of high standard in 9% of cases and poor/insufficient in 41% of cases **(D5.8)**.

Given that "omics" technologies help to elucidate the mechanism of action of a given CHM treatment, we suggest that these studies have to be applied on those CHM treatments whose efficacy has previously been demonstrated. Since most relevant pieces of evidence on efficacy come from clinical trials, it is advisable to start applying "omics" technologies to animal models of diseases for which efficacy has been proven in clinical trials. This would render a number of CHMs reasonably easy to deal with.

We propose the following scheme for applying "omics" to animal studies of CHM **(D5.9)**:



Additionally, we propose the **following workflow for applying "omics"** to animal studies of CHM:

1) To use a CHM proven to be efficacious in an appropriated animal model by gold-standard measurement methods;

2) To assess variability in CHM composition and select a uniform batch;

3) To assess variability in the animal population by carrying out pre-dose omics studies including metabolic profiles;

4) To assess the known levels analytical variation in relation to the changes observed (whether at the transcript, protein or metabolism levels);

5) To define precisely the experimental groups as well as their size in terms of number of animals to made accurate statistical analysis. It will be desirable to choose homogeneous animal population as much as possible;

6) To check that the effects observed in animals after TCM use, especially if chronic administration is necessary, are due to the treatment and not to some other artefact, (e.g. aging or body weight changes, simply not eating because animals feel unwell, changes in gut microflora, etc). Collect as much meta-data as possible in case any of the above differences need to be explained;

7) To perform the appropriated OMIC technique and analysis accordingly to the available guidelines on -omics standardisation in the literature, [e.g. MIAME for transcriptomics, MIAPE for proteomics, SMRS for metabolomics].

Based on common problems identified in publications on CHM animal studies, we have proposed a checklist (Table 3) that could help in preliminary selection of publications lacking the most common problems and thus would be useful for a quick search of reproducible CHM regimens that are likely to be effective in a given context **(D5.10-D5.12)**. The second application of this checklist is to help avoid the most common problems when designing experiments **(D5.11)**.

Table 3. Checklist of methodological aspects of a series of scientific articles on CHM in animal models published in English in the last 10 years (n= 77) in some major areas of diseases

ANIMAL STUDY QUALITY	**TOTAL**	
EXPERIMENTAL DESIGN	**n=77**	**%**
ADMINISTRATION GAVAGE / IV	42	66.67
CONVENTIONAL MEDICINE	12	19.05
RANDOMIZATION	41	65.08
BLINDING	1	1.59
EUTHANASIA PROCESS DESCRIBED	17	26.98
ETHICAL APROVAL GRANTED	30	47.62
BOTANICAL SOURCE[a]	**n=48**	**%**
AUTHENTICATION	16	33.33
VOUCHER SPECIMEN IN OFFICIAL HERBARIUM	2	4.17
PHYSICAL PARAMETERS[b]	1	2.08
PROFILE THE PLANT MATERIAL, AND THE EXTRACT APPLIED[c]	7	14.58
ANALYSIS FOR MICROBIOLOGICAL CONTAMINATION	0	0.00
PESTICIDE RESIDUE ANALYSIS	1	2.08
HEAVY METAL ANALYSIS	1	2.08
PROCESSING	**n=77**	**%**
HARVESTING, POST HARVEST TREATMENT AND DRYING METHOD[d]		
CLEANING	2	2.60
CUTTING/GRINDING FRESH/DRY MATERIAL	1	1.30
FURTHER TREATMENT[e]	1	1.30
STORAGE	1	1.30
EXTRACTION	**n=77**	**%**
PROCEDURE, SOLVENT or PREPARATION OF TRADITIONALLY USED FORMULATION	35	45.45
CHEMICAL CHARACTERIZATION SAMPLE APPLIED[f]	10	12.99

[a] 29 out of the 77 articles analyzed use commercially available proprietary products.
[b] Physical tests include organoleptic evaluation (sensory characters such as taste, appearance, odor, feel of the drug, etc.), viscosity, moisture content, pH, disintegration time, friability, hardness, flowability, sedimentation, and ash value (Anon, 2000)
[c] Chromatographic/spectroscopic targeted analysis in case of known actives, or otherwise non-targeted metabolomics
[d] Heat, sun…
[e] e.g. roasting, etc; traditional additives (honey, sugar, acid, ash, ….)
[f] Unless cut in fresh or dry form, crude traditional Chinese drugs should be moistened to soften the drug prior to cutting.

Worth to mentioning, most problems identified here are not specific of CHM, as shown by previous surveys of publications describing animal research and assessing specific aspects of

experimental design, statistical analysis and reporting (see for instance Kilkenny et al, 2009[2]). But, in view of the need of scientific proof of efficacy, we strongly recommend for future animal studies of CHM **(D5.11 and D5.12)** i) adherence to the recently published ARRIVE (Animal Research: Reporting *In Vivo* Experiments) guidelines (Kilkenny et al, 2010[3]) and ii) the use of specific guidelines for particular diseases (i.e., Workman et al, 2010 for animal studies of cancer[4]).

1.7 WP5 plans for the next months (during the extended lifespan of the FP7 GP-TCM project):

Regarding WP5 plans for the next future, we are about to present a Ph.D. work of one of our members (Ms. Noelia Tejedor). We also are working on a manuscript led by Dr. Xiaodong Cheng on animal studies of cancer in CHM. Finally, we will explore opportunities for new grants in CHM.

1.8 WP suggestions to the GP-TCM Research Association regarding the future of the area covered by WP5:

We propose a committee on Animal studies within the new Association to continue the activities of WP5 expert network under a new roof.

1.9 WP5 Finances:

With a total budget of 39,590 € (UA) plus 3,210 € (SERMAS), we have spent 8,027.76 € in the kick-off meeting and 4,449.16 € to attend to the 11th meeting of the International Society of Ethnopharmacology to present the results of WP5 work (3 communications). Remaining budget will be used to cover activities described in item 7.

1.10 Final Conclusions of WP5:
A panel of experts reviewed bibliography on CHM in animal models and delivered reflexions in several documents. These documents have been considered as valuable material to build most WP5 deliverables and the review "MEDLINE-based assessment of animal studies on Chinese herbal medicine" (published in J Ethnopharmacol). On the other hand, several issues addressed in the preparation of D5.9 (Recommendations on the application of functional genomic studies and chemical synthesis to the study of CHM in animal models of disease) were shared with WP4. The conclusions of this colaboration were published in J Ethnopharmacol (Title of the review "Omics techniques in systems biology approaches to Traditional Chinese Medicine research: present and future").

1.11 Discussion session (Led by Dr. Qihe Xu)**:**

- TCM is a human-based medicine. What's the role for animal studies in modern TCM research?
- Animal studies are designed based on either clinical evidence (for mechanisms) or in vitro studies (for establishing in vivo concept). Where are these compelling clinical and in vitro evidences, especially in Europe, in support of further animal studies?

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[2] Kilkenny C, Parsons N, Kadyszewski E, Festing MFW, Cuthill IC, et al. (2009) Survey of the Quality of Experimental Design, Statistical Analysis and Reporting of Research Using Animals. PLoS ONE 4(11): e7824. doi:10.1371/journal.pone.0007824

[3] Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010). Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 2010 Jun 29;8(6):e1000412.

[4] Workman P, Aboagye EO, Balkwill F, Balmain A, Bruder G, Chaplin DJ, Double JA, Everitt J, Farningham DA, Glennie MJ, Kelland LR, Robinson V, Stratford IJ, Tozer GM, Watson S, Wedge SR, Eccles SA (2010). Guidelines for the welfare and use of animals in cancer research. Br J Cancer.102:1555-77.

- Can TCM diagnosis in patients applied to animal models?

Minutes and discussions at the GP-TCM Final Conference:

WP5 Session on Functional Genomics in Animal Studies – Xuebin Dong (XD) (KCL)

The Session was chaired by Qihe Xu (QX). He introduced the history of the formation of WP5 when the GP-TCM grant was first prepared and the reorganisation of the WP membership after funding was awarded. In particular, due to pressing commitments at work and personal reasons, WP5 coordinator Javier Lucio-Cazaña and Assistant Coordinator María Laura García Bermejo could not attend the meeting. They produced the WP5 report and slides, which were presented by **XD**, who was recently appointed WP5 deputy coordinator.

XD thanked **QX** for the introduction and gave credit of his report to Javier, Laura and all WP5 members. Especially, he paid attribute to the late Professor Sue Watson, who used to serve as Deputy Coordinator of WP5.

XD's report covered the following aspects:

- A brief introduction to WP5 objectives
- Deliverables
- A brief history of WP5 membership and leadership development
- Year 3 WP5 activities
- Year 3 deliverables
- Year 3 experiences gained and lessons
- WP5 summary to Year 1-3
- WP5 plans for future activities towards the extended lifespan of the FP7 GP-TCM project
- WP suggestions to the GP-TCM Research Association regarding the future of the area covered by WP5
- WP Finances
- Final Conclusions of WP5

WP5 discussions – Qihe Xu (KCL)

QX listed the following issues to be considered for discussion.

- TCM is a human-based medicine. What's the role for animal studies in modern TCM research?
- Animal studies are designed based on either clinical evidence (for mechanisms) or in vitro studies (for establishing in vivo concept). Where are these compelling clinical and in vitro evidences, especially in Europe, in support of further animal studies?
- Can TCM diagnosis in patients applied to animal models?

Geoffrey Burnstock **(GB)** raised his concern about the lack of clear conclusions in WP5 report. **QX** acknowledged that **GB's** comments were very important. Indeed, while giving the statistics of literature analysis and identifying problems were certainly important, giving conclusions in a positive light is also very important. For example, the WP5 papers for the JEP Special Issue did not stop at concluding on the poor quality of research materials and research design in animal studies of CHM, it went further to provide a checklist to guide readers to collect important data to ensure quality of research materials and research design. In addition, WP5 research found only 5% papers observe not only the positive side (therapeutic values) of CHM, but also negative side (side effects, mainly merely reporting body weight). This has informed the needs for integrated training in quality, toxicology and pharmacology and this has contributed to the innovative training strategies of the FP7 Marie Curie ITN applications in the past three years. In addition, for other conclusions of the WP, QX recommended attendees to

read the WP4/WP5 paper on Omics application in CHM research and the WP5 paper on animal studies of CHM.

Vivian Wong and De-an Guo both emphasised that animal studies are a very important area in CHM studies and much more could be done in the future.