Spontaneous tumours in dogs

Nosology convergence for 4 tumour types

15-16 January 2014

ONIRIS Nantes & Nantes Events Centre
WELCOME SESSION

Fabien Calvo introduced the meeting and recalled that one of the Measures of the Cancer Plan (2009-2013) was to support the development of spontaneous tumour models and alternatives to clinical trials.

The objective were to increase collaboration between veterinarians, researchers and clinicians so as to promote the translation of results from cancer research, through support of research projects and development of tools to monitor treatment of spontaneous cancers in animals that are diagnosed and treated in a way to be a potential outlook for humans.

Therefore, from 2009 on, INCa redoubled its efforts to mobilize the different research communities (biologists, clinicians and veterinarians) through specific seminars and meetings. Collaborations were established with European partners, and cooperation put in place with the US Comparative Oncology Trials Consortium.

It was decided to launch for the first time in 2011, and renewed in 2012 and 2013, dedicated calls for proposals with the objectives to encourage the various disciplines to work together and incorporate research carried out on spontaneous cancers occurring in animals, particularly dogs, into studies done on a worldwide scale in the areas of biology, diagnosis, prognosis and treatment of cancers that affect humans.

As a result, 8 projects were selected and funded for a total amount of 2.5 M€ since 2011 (see figures under)
The meeting in Nantes had the objectives to:

- Foster discussion and drive collaborations between clinicians in veterinary and human medicine to expedite translation into clinical practice in comparative oncology.

- Focus, from spontaneous tumors in dogs, on nosology convergence for 4 tumor types: breast, lymphoma, melanoma and sarcoma.

**Introduction by Fabien Calvo** (PDF)

**Jérôme Abadie** described the strengths and infrastructures of veterinary medicine in France. Like the human medicine it is established with clinical care units and veterinary specialties.

It is then organized as follows:

- 4 veterinary schools (Nantes, Maisons-Alfort, Lyon and Toulouse), 2 of them with specialized oncology departments (Lyon, Nantes) and associated research units dedicated to oncology.

- 2 private clinics dedicated to oncology: MICEN VET (Créteil) and ONCOVET (Lille) working closely with the Lille SIRIC.

- 1 canine genetic research unit who developed 1 national canine biobank (Cani-DNA) (CNRS, Rennes).
J Abadie also presented the AMAROC Research Unit based in Nantes (research and preclinical investigation in oncology on spontaneous canine and feline cancers) that brings together veterinarians and clinicians. The research projects in AMaROC team dedicated to spontaneous models of cancers are focused on:

I) the study of spontaneous cancers in animals and of their relevance as models for comparative oncology. This axis is mainly dedicated to cancer in dogs and cats, with special interest in cancer types selected for their homology with human ones and for their high frequency in pets: mammary carcinomas, malignant lymphomas, osteosarcomas, melanomas and gliomas. Ongoing projects cover comparative histopathology, immunohistochemical classification studies and molecular characterization, with special emphasis on subtypes of mammary carcinomas and B-cell lymphomas with a translational interest and on therapeutic targets. Collaborative studies are also ongoing concerning influence of environmental contaminants on animal spontaneous cancers (metabolomic studies and contaminants detection by mass spectrometry)

II) the application of above-cited models to cognitive and preclinical studies in oncology, with as examples:
- Innovative protocols for nuclear imaging and radioimmunotherapy in diffuse large B-cell lymphoma (explored in a canine spontaneous lymphoma model of DLBCL).
- Identification of new predictive factors for therapeutic responses in mammary carcinomas (explored in canine and feline spontaneous “triple negative” mammary carcinoma models)
- Study of pharmacokinetics and of in vivo biological effect of innovative pharmaceutical compounds in dogs and cats with spontaneous mammary cancers (in collaboration with pharmaceutical companies)
- Preclinical evaluation of an innovative internal radiotherapy of glioma in a large spontaneous animal model (dog) (lipid nanocapsules loaded with Rhenium-188)

All these studies are developed in collaboration with research units of the Cancéropole Grand-Ouest, UMR 892 Inserm-CNRS (Centre de Recherche en Cancérologie Nantes-Angers), UMR-S 1066 Inserm MINT (Angers), Institut de Cancérologie de l’Ouest, GIP ARRONAX.

- State of the art France J Abadie (PDF)

Catherine André described the French long term collaboration between the “canine Genetics” team at CNRS Rennes, veterinarians and clinicians and work done on comparative oncology. She presented the efforts performed by the team for 20 years on the canine genome and canine genetics, the objectives being to provide relevant canine models of cancer and to propose predictive markers and therapeutic targets. Since 2000, the team developed a network of vets including the vet Schools, Antagene company, French veterinarians, histopathology labs, cancer specialized centres ... and based on that, created a National canine biobank (Cani-DNA). The aims of the biobank are to collect dog samples with clinical and genealogical data, to prepare DNA and RNA, archive and quality control the samples and to distribute samples for dog and human biomedical studies. To date the biobank contains 12 000 DNA and 2000 tissue samples of over 100 genetic diseases mainly cancers.

- CANI-DNA Biobank (PDF)
Stefano Comazzi gave an overview of the situation in Europe. He mentioned that in terms of collaboration in comparative oncology at the European level, there is no funded projects so far. However, several applications were done in the last years mainly for COST actions. There are also some specific collaboration projects born in the context of scientific societies such as the European Society of Veterinary Oncology and the Veterinary Society of Surgical Oncology.

S Comazzi focused on the experiences they are currently making with the European Canine Lymphoma network that took a great part of his last two years work. He ended with examples of ongoing collaborations on some specific issues that stress the need of a collaboration task force at European level to reach high quality results.

Next important dates:

- May 2014 at the annual meeting of ESVONC
- October 2014 in Milan in conjunction with the meeting of the European society of veterinary clinical pathology
- June 2015 in Lugano: 13th International Conference on Malignant Lymphoma

S. Comazzi provided potential directions for the future:

First, the necessity of spreading the collaboration between scientists involved in research, diagnosis and therapy of canine lymphoma (and of other cancers) in order to standardize procedures and define common guidelines for therapy and diagnosis.

Second, there is a need to identify some specific issues to be addressed for cooperative collaboration between different institutions and using different skills and technologies.

- State of the art Europe S Comazzi (PDF)

Chand Khanna first mentioned that the intramural programme (COP) was staffed with a handful of people and that the extramural programme was organized as a network gathering 20 vet schools in the US. The programme is very much focused on drug development rather than biology. Chand Khanna pointed out that collaboration in comparative oncology can be summarized by: “if you have a question on the human, can we have the same question for the canine model and can we answer it?” There is a need that clinicians and veterinarians work in close collaboration and that comparative oncology answers very specific questions.

Khanna mentioned the need to have support from pharmas to develop early phase trials with novel cancer therapeutics in dogs. Funding can be provided by institutional bodies or pharmas.

The issue is that studies on animals are not integrated in the human development. Studies will not go ahead after phase 1 in humans, whereas, a trial in dogs should occur at each step of the clinical development in human.
Chand Khanna advocated for an integrated approach that could have several very useful applications to speed up human clinical development:

- to eliminate rapidly drugs without going further in the clinical development, which allows a cost saving;
- to adopt the “pick the winner” strategy by performing preclinical comparisons of several molecules in order to choose the best one to enter into human clinical development;
- To contribute to dose determination. The dose determination in dogs can help to find the dose in humans. A PK study can be performed in dogs and have several dosing times and the results will help to determine when dosing in human.
- To perform translational research on biopsies from dogs clinical trials;
- To provide appropriate models in adjuvant setting where drugs are active against micrometastases and not on the primary tumour. It takes a very long time to evaluate the efficacy of these treatments in humans and dog models could shorten the required timescale.
- To shrink toxicities studies.

Comparative oncology is a good model for paediatric cancers and/or rare tumours; indeed FDA would accept non clinical trials and results in canine models to allow testing the drug in very few human patients. There is an opportunity to get a priority list of drugs to be tested.

- State of the art USA C Khanna (PDF)

**PARALLEL SESSIONS**

Attendees joined the different parallel sessions focused on specific tumours:

- Melanoma chaired by C. André and E. Maubec
- Breast carcinoma chaired by J. Abadie and M. Campone
- Sarcoma chaired by C. Khanna and JY. Blay
- Lymphoma chaired by P. Devauchelle and S. Le Gouill
Presentation of Aviesan-ITMO cancer funded projects on spontaneous tumours models in animals

Catherine ANDRE

Canine spontaneous melanoma: Comparative genetic and mechanistic analyses of human and canine melanoma pathways

Presentation of the different work packages:

Investigate the genetic bases of canine melanoma
- Predisposition and tumour progression
Status: Ongoing

Comparison with human melanomas
- explore the similarities at the histological level
Status: Done on 150 Cases / Ongoing on 300 cases
- explore the mechanistic aspects of non-UV pathways
Status: To be done

Transfer the results obtained in dogs by screening alterations in the homologous human genes, gene families and pathways
Status: Ongoing: retrospective and prospective human sample collection

Catherine André concluded that dog melanoma is relevant models for non UV-linked human melanomas:

- mucosal melanomas (frequent in dogs)
- acral and rare cutaneous melanomas of dermal origin (frequent in dogs).

There is an opportunity to decipher the «non UV pathways» of melanomas and the role of pigmentation genes in melanoma.

RNAseq and exome sequencing analyses of paired samples are ongoing with the objective of a better characterization of molecular alteration and the identification of new therapeutical targets

François DAVODEAU

-Preclinical trial of radioimmunotherapy of spontaneous Diffuse Large B-Cell Lymphoma (DLBCL) in the dog model

The CANIMAB project is divided in 3 work packages:

WP1 – Comparative oncology of human and canine DLBCL
WP2 – Generation of antibodies specific for canine lymphomas
WP3 – the canine spontaneous b-cell lymphoma as preclinical model for nuclear imaging
Preclinical trial of radioimmunotherapy of spontaneous Diffuse Large B-Cell Lymphoma (DLBCL) in the dog model (PDF)

-Monoclonal antibodies for Imaging and Radioimmunotherapy of B-cell lymphoma in Small Animals

The objectives of the MIRSA project are:
WP 1 – characterization of antibodies against cd20 and cd22 in term of pharmacokinetics parameters
WP 2 – spontaneous canine diffuse large b-cell lymphoma as a preclinical model for nuclear imaging

In conclusion:
-The counterpart of human DLBCL has been identified in dog
-Monoclonal antibodies against CD22 are available for clinical trial in dog
-Multicentre evaluation of immuno-SPECT is programmed
-Evaluation of immuno-TEP will be performed thanks to ICO gift of PET-CT camera
-Tools for pharmacokinetics studies in dogs will be soon produced
-Availability of new isotopes thanks to ARRONAX cyclotron

Catherine THIEBLEMONT

-Develop dog-ized antibodies for translational cancer research on lymphomas

The aims of the project are to better characterize the lymphoma types in dogs as compared to human lymphoma classification at the molecular level and to develop Canine Specific Antibodies Against Cancer Associated Targets, to identify Novel clinically relevant canine cancer targets and determine if spontaneous tumours dogs models could be useful to identify clinically relevant targets for human therapy.

Georges VASSAUX

-Towards an oncolytic vaccinia virus targeting high-grade breast carcinoma.

The project is an in vitro approach to compare the efficacy of an oncolytic vaccinia virus on primary canine breast carcinoma cells from tumours of different grades.
Partial conclusion is that Oncolytic VVtk- has a very significant oncolytic activity on different subtypes of breast carcinomas, Grade 3 primary breast cancer cells are less responsive than primary cells from other grades.

Georges Vassaux presented OncoBioTek which is a comparative oncology platform located in the South East of France. OncoBioTek develops among other things primary cell culture-based in vitro models for the efficient screening and validation of novel anti-cancer treatments.

Georges Vassaux explained that spontaneous tumours in dogs could be the missing link for the development of new oncolytic viruses. Indeed the stages of development are currently as follows:

- Genetic design of the virus
- Tests on tissue cultures
- Tests on established tumours in mice
- Human clinical development

In conclusion, the future could be to conduct veterinary clinical trials to test new oncolytic viruses to provide a unique platform to tailor these therapeutic agents to incurable diseases in humans.

- [Towards an oncolytic vaccinia virus targeting high-grade breast carcinoma](#) (PDF)

**Fanny BLANC**

*Immunity to regressive melanoma in a swine spontaneous model of cutaneous melanoma.*

In this specific model, the animal exhibits naturally occurring melanomas that regress completely 6 months after birth. To understand the mechanisms would be of help to progress in this pathology.

Presentation of the different parts of the project which are:
- Identification of the cellular and molecular immune actors in the course of melanoma outcome
- Functional study of immune response against melanoma cells
- Effect of chemotherapy on immune response against melanoma

- [Immunity to regressive melanoma in a swine spontaneous model of cutaneous melanoma](#) (PDF)
FEEDBACK FROM DAY 1 PARALLEL SESSIONS:

Breast carcinoma:
Since 2008, the Orpheo group conducts translational research on spontaneous animal models of breast cancers. The objective is to validate the coherence between cats/dogs and human breast tumours. 350 canine invasive breast tumours were collected and IHC analyses were performed on these tumours by 4 vet pathologists and 1 human pathologist.

77% of canine breast tumours are triple negative.

As these dogs are only treated by tumour resection, this cohort is very useful to better understand the natural history of the disease, which is impossible now for human patients.

There is still a need to confirm that the phenotypic aspect is correlated with molecular characteristics of these tumours.

Discussion went also on obesity and cancer. Is dog a validated model to establish a link between obesity and cancer? What is the role of the IgF-1R?

During the session, discussion focused on the need to:
- strengthen the link between CLIP² and veterinarians
- develop the interest of pharmas to have comparative oncology tests to have access to new drugs
- conduct multicentric trials (Europe)

Lymphoma:
One of the next objectives could be to establish the same treatment’s algorithm (parallel to the Han’s algorithm) in dog and human with common prognostic factors.

Some studies have been already done in dog, for example study of the c Kit mutation in adult, children and dog for the treatment of the mastocytosis.

Lymphoma is a good model and is ready for clinical trials. Teams are also ready to go.
Sarcoma:

In osteosarcoma, there are genetic, biological and histopathological similarities between dogs and humans that favour comparative studies towards drug development.

In soft tissue sarcoma, diverse groups are represented, at every age of patient, with a polylocalisation.

For sarcomas we could conclude that i) there are rare and multiple heterogeneous subtypes and molecular subtypes ii) Treatment adapted to driving molecular alterations in 20-25% of the patients only iii) on driver mutation there could be work done with dogs.

In histiocytic sarcoma, specific breeds are strongly predisposed and constitute interesting models to decipher the genetic predisposition of this rare cancer in humans. There are different clinical forms homologous to specific human forms: it is easier to analyse the genetics in these dog breeds to provide candidate therapeutic targets in human. Histiocytic sarcoma pathways are being analysed in dogs to transfer to human histiocytic disorders.

The issue is to know how comfortable and interested will be a clinician if a sarcoma provided by the dog spontaneous model is not exactly the same. But this would be an approach for a proof of concept and shows the importance to well characterize the extent of homology of the different cancer types between human and dogs.

Melanoma:

Dog melanomas are relevant models for non UV-linked human pathways of melanomas (especially for acral and mucosal human melanomas).

Comparative genomic studies between human, pig and dog are ongoing (INCa PLbio funding).

Based on results already obtained, New approaches could be:

- PI3K pathway would be relevant to study as we don’t observe BRAF mutations in dogs melanomas.
- To explore immunotherapy in dogs.

Epidemiology E Maubec (PDF)
GENERAL DISCUSSION (ALL) AND CONCLUSION (FRÉDÉRIQUE NOWAK, INCA):

1. Some key points were highlighted during this meeting:

   - There is a clear added value of using spontaneous tumours in pet dogs models for the clinical development of human drugs, more specifically to:
     - “Pick the winner”: generate additional scientific data from pet dogs clinical studies to choose among several candidate drugs the best one for clinical development;
     - Evaluate drugs in adjuvant setting;
     - Shorten early phases clinical trials
     - Perform translational research on dog biopsies enrolled in a clinical trial through the gathering of serial biopsies.

   - Some pet dogs cancers share significant similarities with human cancers both at histological and molecular levels and can be used right now for human drugs development:
     - Triple negative breast cancer;
     - Lymphoma (DLBCL);
     - Osteosarcoma;
     - Histiocytic sarcoma;
     - Non UV-linked melanoma.

   - A close collaboration between veterinarians and “human” clinicians in order to answer specific questions and get needed answers is key for success.

2. Some needs were identified during this meeting:

   - At the national level:
     - There is a need for structuring through:
       - The creation of a network of pet dogs clinical trials centers. Note: there are only 3 out of the 4 French veterinarian schools and 2 private clinics that treat cancer.
The implementation of direct and formal links between veterinarians and human clinicians conducting early phase clinical trials (CLIP²). A French Comparative Oncology Consortium (Société Française d’Oncologie Comparative) could be created under the umbrella of INCa. It would gather veterinarians and human clinicians interested in comparative oncology and would not focus on a specific pathology.

The implementation of specific links with cooperative groups.

- There is a need for training veterinarians on the methodology to conduct high quality clinical trials in dogs. Human clinicians conducting early phase clinical trials should be involved in it and it could be one of the tasks of the French Comparative Oncology Consortium.

- There is a need to inform human clinicians and pharmaceutical companies on the added value of using comparative oncology for human drug development and on the availability of reliable pet dogs’ spontaneous tumours models. A letter summarizing the conclusions of this meeting could be submitted to JNCI.

At the international level:

- There is a need for structuring a European network of people interested in comparative oncology. The best way to do this has yet to be decided (new COST application, Horizon 2020, other...)

- Cooperation with NCI could be set up. Chand Khanna proposed to give European Comparative Oncology teams access to the NCI comparative oncology infrastructures.