Quality control & quality assurance of canine biological specimens available through the Pfizer-CCOGC biospecimen repository for comparative oncology studies

Rachael Thomas [1,2], Mark Simpson [3], Hiroyuki Mochizuki [1], Christina Williams [1], Kelsey Poorman [1], Katie Kennedy [1], Christina Mazcko [4], Jaime F. Modiano [5,6] and Matthew Breen [1,2,7]

[1] Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC; [2] Center for Comparative Medicine and Translational Research, North Carolina State University, Raleigh, NC; [3] Laboratory of Cancer Biology and Genetics & [4] Comparative Oncology Program, Center for Cancer Research, National Cancer Institute, Bethesda, MA; [5] Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN; [6] Masonic Cancer Center, University of Minnesota, Minneapolis, MN; [7] Cancer Genetics, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill

Introduction: the CCOGC

The Canine Comparative Oncology and Genomics Consortium (CCOGC) was conceptualized in 2004 to reflect growing interest in parallel studies of canine and human cancer genomics.

In 2007 the CCOGC incorporated as a 'not for profit' entity with 501(c)3 status, with a lead gift from Pfizer Animal Health and substantial donations from the American Kennel Club Canine Health Foundation and Morris Animal Foundation.

The primary aims of the CCOGC are [1]:

- to develop opportunities arising from a more advanced understanding of the genetics and biology of companion animal cancers
- to guide the development of novel technologies, and to permit the integration of appropriate canine cancers in the global study of cancer biology and therapy
- to facilitate strategic partnerships and collaborations across diverse disciplines in canine and comparative cancer biology, and aid sharing of resources and reagents
- to develop a highly-annotated biospecimen repository to support these aims

In 2007, the CCOGC initiated collection of biospecimens from naturally-occurring primary canine cancers across all dog breeds in eight major veterinary institutions within the USA.

To date the Pfizer-CCOGC Biospecimen Repository has recruited 60,956 specimens from almost 2000 canine cancer patients (Figure 1) across seven major cancers that represent the greatest impact on canine health and welfare, with maximum comparative and translational relevance.

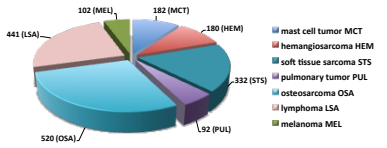

Figure 1: Distribution of cases submitted to the Pfizer-CCOGC Biospecimen Repository to date, according to the seven primary tumor histologies.
Recruitment was focused on three histologies with extensive comparative relevance (lymphoma, osteosarcoma and melanoma). Note abbreviations used for each tumor histology.

This report describes a quality control and assurance assessment performed on the Pfizer-CCOGC Biospecimen Repository prior to releasing specimens to the scientific community in October 2012.

Pfizer-CCOGC biospecimen collection procedures

All specimens contained with the repository are collected using standardized institutionally approved protocols with informed owner consent, prior to initiation of chemotherapy.

The following specimens are collected from each case and are processed within one hour:

- tumor tissue (snap-frozen, and both formalin- and OCT-fixed)
- healthy tissue (snap-frozen, and both formalin- and OCT-fixed)
- peripheral blood
- serum
- plasma
- urine

Each submission Is associated with the following patient data:

- age
- breed
- neuter status
- weight
- gender
- collection date
- tumor diagnosis
- tumor subcategory
- pathology report

Specimens are de-identified at source, divided into representative replicates as required, barcoded and recorded in a central, real-time 'Tissue Tracker' relational database along with associated clinical history, prior to transfer to the Biospecimen Repository, managed by Fisher Bioservices and housed within the National Cancer Institute (NCI) Frederick Central Repository Services.

Study aims

This internally commissioned study was performed to evaluate the quality of the Pfizer-CCOGC Biospecimen Repository prior to commencing sample withdrawal by the scientific community.

Quality control and assurance parameters were assessed on a panel of biospecimens distributed randomly across the eight submitting institutions and the seven tumor histologies represented in the repository:

1) To perform a detailed pathology review of the tumor histology of selected biospecimens
2) To assess the quality and yield of nucleic acid obtained from tumor tissue and peripheral blood, as the primary reagents destined for downstream comparative genomics studies

Pathology re-review of biospecimens

A total of 331 formalin-fixed and paraffin-embedded (FFPE) tumor specimens, representing a cross-section of all seven tumor histologies, was subjected to rigorous pathology re-review by a panel of board-certified veterinary pathologists at the NCI.

H&E-stained specimens were i) evaluated in context with the original histologic diagnosis assigned by the submitting veterinarian, and ii) assessed for tumor versus stromal contamination by routine histopathology, immunohistochemistry and quantitative morphometry [2].

Outcome of pathology evaluation

The proportion of cases for which the original diagnosis was corroborated ranged from 64% (hemangiosarcoma) to 100% (lymphoma and osteosarcoma), with a mean of 89% across all cases. An alternate diagnosis was reached for 6% of all specimens, and the remaining 5% of specimens showed no clear evidence of a neoplastic process. The low value for hemangiosarcoma was due primarily to lack of sufficient tumor tissue in the submitted specimen to permit conclusive diagnosis.

Table 1: Outcome of pathology re-review of 331 cases, distributed across each of the seven tumor histologies.

Tumor	Number of cases assessed	Number (%) of diagnoses corroborated
MCT	50	47 (94%)
HEM	50	32 (64%)
STS	49	44 (90%)
PUL	50	46 (92%)
OSA	42	42 (100%)
LSA	49	49 (100%)
MEL	41	35 (85%)
TOTAL	331	295 (89%)

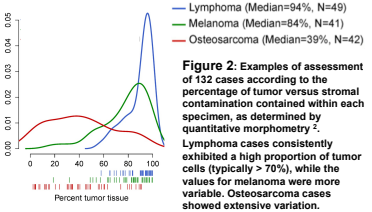

Figure 2: Examples of assessment of 132 cases according to the percentage of tumor versus stromal contamination contained within each specimen, as determined by quantitative morphometry [2].
Lymphoma cases consistently exhibited a high proportion of tumor cells (typically > 70%), while the values for melanoma were more variable. Osteosarcoma cases showed extensive variation.

Acquisition of biospecimens for nucleic acid assessment

A total of 188 cases were selected at random from the biospecimen repository, providing proportional representation of each tumor histology and submitting institution (Figure 3).

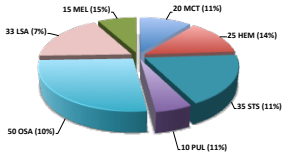

Figure 3: Distribution by tumor histology of the 188 unique cases selected for nucleic acid quality assessment.
Numbers in parentheses indicate the proportion of samples assessed for each histology relative to the total number of samples of that histology available within the biospecimen repository.

For each case the following specimens were shipped on dry ice from the repository (total = 564)

i) one aliquot of unfixed tumor tissue ➜ destined for DNA isolation
ii) a second, replicate aliquot of unfixed tumor tissue ➜ destined for RNA isolation
iii) one aliquot of peripheral blood ➜ destined for DNA isolation

Nucleic acid isolation from biospecimens

Nucleic acid isolation was performed using routine procedures and commercially supplied kits.

Tumor DNA: Tumor tissue (25mg) was first digested to completion with proteinase K, then processed using a Qiagen DNeasy Blood and Tissue Kit with the suppliers' recommended protocol, including the optional RNA-elimination step.

Tumor RNA: Tissue homogenization (25mg) was performed by mechanical disruption using a MiniBeadBeater-24 (Biospec, Bartlesville, OK) equipped with 1mm-diameter zirconia-silica beads. The homogenate was processed using a Qiagen RNeasy Plus Kit as recommended.

Blood DNA: Whole blood (0.2ml preserved in EDTA) was processed using a Qiagen UNeasy 96 Blood and Tissue Kit as recommended, including the optional RNA-elimination step.

Nucleic acid samples were eluted with ultrapure water. DNA yield and quality was assessed by spectrophotometry (Nanodrop) and routine 2% agarose gel electrophoresis. RNA yield and quality was assessed by spectrophotometry (Nanodrop) and by evaluation with an Agilent 2100 Bioanalyzer to determine the RNA Integrity Number (RIN).

The quality of each nucleic acid sample was then scored according to the following three parameters (maximum score for each parameter = 3). These scores were then summed to yield a global quality score for each specimen (maximum global quality score = 9).

Table 2: Summary of parameters used for assignment of quality scores to nucleic acid specimens.

Nucleic acid	Quantity processed	Parameter	Score = 3	Score = 2	Score = 1
Tumor DNA	25mg	Yield	> 10µg	2.5 - 10µg	< 2.5µg
		260:280 value	> 1.8	1.5 - 1.8	< 1.5
		Gel integrity	High molecular weight	Minor degradation	Marked degradation
Tumor RNA	25mg	Yield	> 10µg	5 - 10µg	< 5µg
		260:280 value	> 2.0	1.6 - 2.0	< 1.6
		RIN	> 8	6 - 8	< 6
Blood DNA	0.2ml	Yield	> 3µg	1.5 - 3µg	< 1.5µg
		260:280 value	> 1.7	1.5 - 1.7	< 1.5
		Gel integrity	High molecular weight	Minor degradation	Marked degradation

Outcome of nucleic acid quality assessment

All 564 biospecimens were processed successfully to generate a quantifiable nucleic acid specimen, for which a yield and quality score could be assigned (summarized in Figure 4).

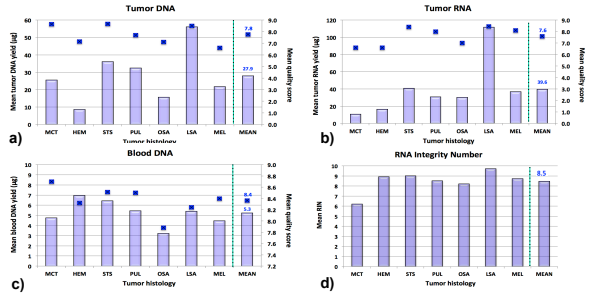

Figure 4: Assessment of nucleic acid quality across 188 tumor specimens, categorized by tumor histology. In charts a-c), blue bars indicate the mean yield of nucleic acid obtained from specimens within each cancer, and the blue squares indicate the mean quality score assigned to those nucleic acid samples. RIN values associated with RNA samples are summarized in d). For each chart, the global mean values across all 188 cases are shown on the far right.

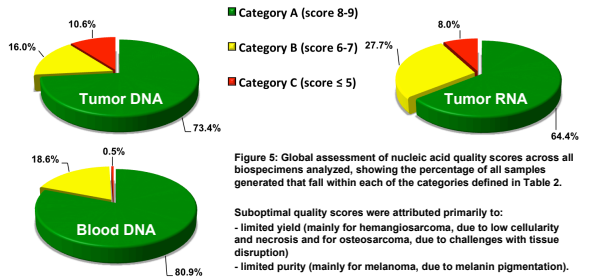

Figure 5: Global assessment of nucleic acid quality scores across all biospecimens analyzed, showing the percentage of all samples generated that fall within each of the categories defined in Table 2.

Suboptimal quality scores were attributed primarily to:
- limited yield (mainly for hemangiosarcoma, due to low cellularity and necrosis and for osteosarcoma, due to challenges with tissue disruption)
- limited purity (mainly for melanoma, due to melanin pigmentation).

Across the panel of 188 cases > 73% of all DNA samples, and 64% of all RNA samples met the criteria for classification as optimal quality samples (category A). The proportion of samples classified as suboptimal (category C) was highest for tumor DNA (10.6%) followed by tumor RNA (8%), with only 0.5% of blood samples falling into the lowest quality category.

Conclusions

Quality control and assessment of randomly selected samples from the Pfizer-CCOGC Biospecimen Repository, representing approximately 10% of all cases submitted, demonstrated:

- The diagnostic classification of almost 89% of submitted specimens was corroborated by in-depth re-review by a panel of independent veterinary pathologists

- The proportion of specimens yielding nucleic acid considered to be of suitable quality for most downstream genomics applications (categories A and B) was 89.4% for tumor DNA, 92.1% for tumor RNA and 99.5% for blood DNA

This evaluation of the Pfizer-CCOGC Biospecimen Repository provides further data to reinforce the potential value of this sample collection for furthering advances in canine and comparative cancer studies.

The repository is now open for access to the scientific community through a peer-reviewed application procedure. Almost 1000 specimens have been released for research studies to date.

For further details contact the CCOGC via http://www.ccogc.net/ or administration@ccogc.net.

References

[1] http://www.ccogc.net/
[2] Webster JD et al., J Biomol Tech. 2011 September; 22(3): 108–118

Acknowledgements

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