Data Submission Request to the Integrated Canine Data Commons

1. Name/Identifier of Study:

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Study name: Clinical and biological effects of the BRAF inhibitor, vemurafenib, in dogs with naturally-occurring invasive urothelial carcinoma harboring the $BRAF^{V595E}$ mutation (dog homologue of the human $BRAF^{V600E}$ mutation).

2. Grant ID and funding source (if applicable):

No federal funding was used in the support of this work. The study was made possible by a grant from the Puppy-Up Foundation, by internal support from Genentech, and through private donations made to Purdue University in support of the work.

3. IACUC/IRB approval numbers (if applicable):

Purdue Animal Care and Use Committee Approval Number: #1111000124

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6. Data access policy (choose one): Open-access – no-embargo, Controlled-access – no

embargo, Open-access – embargo, Controlled-access – embargo

The data will need to be embargoed until the publication is in print. We anticipate submitting the publication by June 2020. We do not mind transferring the data before the publication is submitted, but will need to have the data embargoed until the publication is in print.

7. Cancer type(s) included in study:

Canine urinary bladder cancer, specifically invasive urothelial carcinoma (also referred to as transitional cell carcinoma).

This study is also highly relevant to BRAF-driven cancers in humans including metastatic melanoma, subsets of colon cancer and thyroid cancer, and less commonly cancers arising from other sites. In fact, the BRAF^{V600E} mutation is thought to drive 8% of all human cancer across many cancer types.

8. Number of subjects included in study:

34 dogs with invasive urothelial carcinoma expressing BRAF^{V595E} mutation

9. Data types included in study (check all that apply): Imaging, genomics, proteomics,

immunology, clinical, other (specify)

The 34 dogs in this study participated in a phase I/II trial of vemurafenib. The dogs were scheduled to have cystoscopy and tissue collection immediately prior to the start of vemurafenib treatment, after one month of treatment, and when possible, at the time of relapse. Sufficient samples for RNA-seq analyses were available from: 29 dogs prior to treatment, 25 dogs pre-treatment and at one month into treatment, and 17 dogs at all 3 time points (pre-treatment, one month, relapse).

The types of data include:

- pathology (reports including tumor grade)
- imaging reports
- genomics (RNA-seq, molecular subtype, immune type, BRAF mutation status, pathway analyses)
- immunology (IHC for CD3 positive infiltrating lymphocytes, RNA-seq patterns defining tumors as immune "hot" i.e. immune infiltrated vs "cold" i.e. non-immune infiltrated)
- pharmacokinetic data
- maximum tolerated does (MTD) data

- clinical data (breed, sex, neuter status, date of birth, date of diagnosis, age at diagnosis, TNM stage at diagnosis and at death, treatment summary including tumor response and PFI, adverse events, treatment after the trial, date of death, age at death, survival, necropsy findings, cause of death)

Also, please note, limited frozen tissue samples are available from dogs in the trial, and our group could submit these for WES or WGS in the future. This would not be part of this current submission to ICDC, but could be added in the future if a mechanism for adding data should become available.

10. Amount of data (in TB):

This will depend on which type of file is preferred: Fastq.gz, fastq, bam?

For Fastq.gz files, approximately 2 TB of memory will be needed. If a different type of file or combinations of files are transferred, this will require more memory.

11. The overall scientific benefit of including this study in the ICDC prototype:

There are at least 2 major scientific contributions of this work.

First, the data set offers extensive information concerning naturally-occurring invasive urothelial carcinoma in dogs. This is thought to be the most clinically relevant animal model of muscle invasive bladder cancer in humans. The data allow further characterization of the model at the molecular level. This is key because 50% of humans with muscle invasive bladder cancer die from it, and animal models are essential to further understand the biology of the disease and to test new treatment strategies with the most promising approaches taken into humans.

Second, the data set focuses specifically on naturally-occurring cancer in dogs that harbors the BRAF^{V595E} mutation. This is the dog homologue for BRAF^{V600E} mutation in humans. It is intriguing that the canine BRAF mutation is identical to the human mutation in the gene involved, the position of the mutation on the gene, and the base substitution. BRAF^{V600E} drives 8% of all human cancer. The canine trial producing the data submitted here has demonstrated that the canine cancer responds similarly to BRAF driven cancer in humans treated with the targeted therapy, vemurafenib. This is important because there are many compelling unanswered questions about the BRAF mutation in humans and especially about targeting it for therapy. It is currently not known why 50% of patients with BRAF driven metastatic melanoma have dramatic remissions, and 50% have little benefit at all. Similarly, the causes for the emergence of resistance to treatment are poorly defined. The data including cystoscopic biopsies collected before treatment, one month into treatment, and at relapse provide an ideal data set to study the mechanisms of drug response and resistance. Regarding another aspect of treatment, the

use of immunotherapy in BRAF driven cancer in humans holds initial promise with impressive and durable remissions, but this treatment approach requires much more study to improve on the limited 20% remission rate. The dog data set will be contributory in the immunotherapy field; intriguing changes in the immune state (immune infiltrated vs nonimmune infiltrated) have been observed in the serial biopsies in dogs in the vemurafenib trial. To appreciate the importance of the dog data set, it should be pointed out that it is very difficult (if not impossible) to experimentally produce BRAF driven cancer in rodents that truly mimics the human condition. Yet, here we have this much needed model in dogs. And we can submit a data set that can be further mined to answer some of the compelling questions listed above. This is expected to be a critical submission to support the true comparative nature of the work made possible through the ICDC.

In addition to the scientific value discussed above, the data set can also serve two very important roles. First, this data set can be used for "trouble shooting" and refining the process of preparing and transferring data, and incorporating the data into the Commons. Second, this is an extremely valuable data set to test methods and approaches in "Big Data" science. Building complex models that incorporate many types of data (genomic, pathologic, clinical, PK, serial collections over time, etc), developing the systems to effectively mine and interpret that data, and developing predictive modeling approaches in related cases are all key in moving the field ahead in the multi-omic and multi-data type era.

12. Any publications associated with this study, if any:

We plan to submit a manuscript to Clinical Cancer Research by June 2020.

13. Time constraints on processing/loading/releasing the data:

We expect to be able to begin transferring the data in March. The data will need to be embargoed until the publication is in print.

14. Data standards used, if any (e.g., SEND):

NA

15. Anticipated budget needed to prepare data set for submission:

If this data set is accepted for submission, could we discuss what the process will involve? The data set has been generated. The clinical, PK, and selected RNA-seq findings have been imported into an Excel file. Should extensive time be required at Purdue to re-format the data,

or for some lengthy transfer process, we may need to request funds to cover the staff's time for this.

The following documentation should be attached to the submission:

1. Data Dictionary:

<u>Vemurafenib Trial:</u>

Study Overview

The work included a phase I/II clinical trial of vemurafenib (Vem) in dogs with naturally-occurring invasive urothelial carcinoma (InvUC). Dose escalation and determination of the maximum tolerated dose (MTD) was followed by expansion of a cohort of dogs treated at the MTD to further assess antitumor activity. The canine clinical trial was conducted at the Purdue University Veterinary Teaching Hospital (PUVTH) following the guidelines and approval of the Purdue Animal Care and Use Committee and the Clinical Review Board. Informed consent in writing from each dog owner was required for the dog to participate in the trial. The dogs lived at home with their families other than the times they were being evaluated at the PUVTH.

Subject Eligibility

Inclusion criteria were: dogs with histologically-diagnosed lower urinary tract InvUC harboring the $BRAF^{V595E}$ mutation, expected survival time of at least 6 weeks, normal liver function, and serum creatinine <2.0 mg/dL (normal reference range 0.5 to 1.5 mg/dL). The expected survival time was based on the health status of the dog as determined by medical history, physical exam, CBC (complete blood cell count), serum biochemical profile, urinalysis, and imaging (thoracic/abdominal radiography, abdominal ultrasonography). Dogs treated with prior therapies were eligible for the trial if cancer progression had been noted in response to prior therapy or if chemotherapy had been discontinued for a minimum of 4 weeks prior to the dogs receiving Vem. Dogs were not allowed to receive any other cancer treatment such as chemotherapy, surgery, radiation therapy, or nonsteroidal anti-inflammatory drugs (also referred to as cyclooxygenase inhibitors or "Cox inhibitors") during the Vem treatment. A washout period of 5 days was required for prior Cox inhibitor use. [Note that Cox inhibitors have antitumor activity against canine InvUC, and therefore could not be administered concurrently during the Vem trial as that would have interfered with interpretation of the Vem effects.]

Treatment and Trial Design

Vem (Vemurafenib, Zelboraf, Genentech, San Francisco, CA) was provided by Genentech. The initial Vem dose (25 mg/kg BID orally) was selected from previous laboratory dog studies (unpublished data, Genentech). Vem was administered with food as this has been shown to enhance drug absorption. Drug tolerability was assessed by the medical history provided by the dog owner, physical exams by the veterinarian, CBCs, and biochemistry panels. Toxicity was graded using VCOG-CTCAE criteria. The Vem dose was increased by 25 mg/kg BID in subsequent cohorts of dogs using a 3+3 trial design. When dose limiting toxicity (DLT, VCOG grade 3 or 4 toxicity) occurred, the cohort was expanded to six dogs, and the dose further adjusted to determine the maximum tolerated dose (MTD; DLT in no more than one of six dogs). With the knowledge that alanine amino transferase (ALT) has increased and liver function remained normal in laboratory dogs and humans receiving Vem, grade 3 abnormalities in ALT in dogs in the trial that were asymptomatic were not considered a DLT. Serial blood samples were collected at multiple time points for pharmacokinetic (PK) analysis on day 1, and at 2 and 4 weeks of Vem treatment. Cystoscopy and tumor tissues collected for RNA sequencing (RNA-seq) analysis was planned prior to treatment, after 1 month of treatment, and when possible, at cancer relapse. Vem concentrations were measured in tumor tissues collected via cystoscopy. To aid in obtaining biopsies from the same part of the tumor on each scoping, the same operator performed each cystoscopy, and the operator was guided by a tumor map made for each dog using detailed ultrasound exam images, and still and video images obtained at the time of each cystoscopy.

In individual dogs, the Vem dose was reduced by 10% if grade 2 toxicity was noted, and by 20% if grade 3 or higher toxicity was noted (as allowed by tablet size). Dogs that experienced cancer progression or unacceptable toxicity following dose adjustments were eligible to receive other therapies off study.

In order to further define antitumor activity, the cohort of dogs receiving the MTD was expanded with the goal to assess antitumor effects in a minimum of 24 dogs treated at the MTD. The antitumor effects of Vem were assessed by cystosonography using a specific standardized protocol, and physical exam including rectal exam with palpation of the urethra and prostate at 4-week intervals. Complete cancer staging (thoracic radiography with left lateral, right lateral, and ventrodorsal projections; abdominal radiography with right lateral and ventrodorsal projections; and complete abdominal ultrasonography) was performed at 8-week intervals. The response in the primary tumor was determined by comparing volume measurements by cystosonography as follows: complete remission (CR, no residual cancer lesions detected), partial remission (PR, ≥50% decrease in tumor volume and no new tumor lesions), stable disease (SD, <50% change in tumor volume and no new tumor lesions), and progressive disease (PD, ≥50% increase in tumor volume or the development of new tumor lesions). Metastatic lesion response was assessed by RECIST criteria. Progression free interval

(PFI) was defined as the time from the start of Vem treatment until PD of local or metastatic lesion(s) occurred. Permission to perform a necropsy was requested at the time of the dog's death (death from cancer or other causes).

Pharmacokinetic Analyses

Plasma pharmacokinetic analysis was conducted in all dogs in the phase I portion of the study. Blood was collected at 1, 3, 6, and 10 hours after the first dose of Vem on Day 1. At 2 weeks and 4 weeks into treatment, samples were collected at these same time points as well as a sample collected just prior to the morning dose. In dogs in the phase II portion of the study Vem concentrations were measured in plasma at 0, 3, and 6 hrs post administration. Tubes were centrifuged, and plasma collected and stored at -80°C until analysis. Vem concentrations were measured in tumor tissues collected at cystoscopy at 4 weeks into treatment and at the time of relapse with concurrent plasma measurement. The pharmacokinetic assays were performed by Drs. Dietrich Tuerck, Liling Liu, and Alan Den at Genentech. We have permission from Genentech to submit this data to the ICDC.

Sample Size and Statistical Considerations

In addition to the number of dogs required to determine the MTD of Vem, a minimum of 24 dogs treated at the MTD were included as this number of subjects has been sufficient to estimate the response rate with anticancer drugs. Stepwise Cox regression analysis was performed to assess the relationship between predictor variables (age, gender, breed, TNM stage, subtype, drug concentrations, toxicities, etc.) and tumor response and PFI, and univariate and multivariate models were applied.

RNA-seq Analyses

RNA-seq was performed on tumor samples to identify pathways related to the antitumor effects observed. Tumor samples were immediately placed in Trizol (Sigma, St Louis, MO) after collection. RNA was isolated and purified using RNeasy (Qiagen, Valencia, CA) following manufacturer's directions. DNase treatment was performed to ensure a pure RNA sample prep. All tumor RNA samples (pre- and post-treatment with Vem) were batched and processed at the same time by Q2 Solutions (Raleigh NC), as contracted by Genentech. The directional RNA-seq libraries were created, validated, and run on Illumina HiSeq system. This yielded > $20x10^6$ clusters per sample with 2x150 base pair reads. The raw sequence was passed through numerous quality control and filtering steps and aligned against canine genome, CanFam3.1 available from the University of California, Santa Cruz genome database.

The RNA-seq data analyses were performed at Purdue University using Strand NGS (Agilent Technologies, Santa Clara, CA). Pairwise comparison between response groups (remission vs stable disease + progressive disease) was used to detect differential expression (fold change ≥2,

p<0.05) of genes in the pre-treatment biopsies. Pairwise comparison was also used to determine changes in gene signature patterns between the pre-treatment samples and the samples collected one month into Vem treatment within the same tumor response group. In additional analyses, samples from each individual dog were evaluated for changes in genes i.e., changes associated with each dog over the first month of treatment, and at relapse. Genes reported in the literature to be impacted by Vem treatment i.e., MEK and ERK (MAPK pathway) and AKT/PI3K pathway were evaluated. The RNA-seq data were also analyzed to assign the tumors to molecular subtypes, as these subtypes are emerging as important drivers of tumor behavior and treatment response in human bladder cancer. The RNA-seq data were also interrogated using panels of genes known to classify human bladder cancer as immune "hot" (infiltrated by T lymphocyte effector cells, pro-immune signatures) or immune "cold" (not infiltrated by T cells, immunosuppressive signatures). Gene ontology (GO) analyses were also conducted on the tumor samples pre-treatment, post-treatment (at 1 month), and at time of relapse.

Assembled Data to Submit to ICDC:

The data include RNA-seq data, the analyses of the RNA-seq data described above, PK data, and clinical data. The clinical data and key findings from other analyses have been assembled in an Excel spreadsheet. The clinical data include: breed, sex, neuter status, date of birth, date of diagnosis, age at diagnosis, TNM stage at diagnosis and at death, treatment summary including tumor response and PFI, adverse event data, date of death, age at death, survival, necropsy findings, and cause of death.

2. Data Model/Schema diagram indicating how collected data relates to subjects,

visits, samples, etc:

The collection and analyses of the data are described above. Each dog was given a unique identifier number linked (in a secure location at Purdue University) to the dog/owner's name and contact information.