Evaluation of palliative therapy, alone or in combination with toceranib phosphate, in dogs diagnosed with metastatic or recurrent beta-cell neoplasia

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Supplementary Information 1. Equipment and methodology used for measurement of insulin at diagnosis, monitoring after surgery and for monitoring dogs during the study for clinical signs of hypoglycaemia and adverse events.

The haematological analyses were performed using a standard haematology analyser (ADVIA 120, Siemens Healthcare S.L.U., Madrid, Spain). The biochemical profile was constructed using reflection spectrophotometry (Refrovet Plus, Scil animal care company, Viernhheim, Germany) for glucose, creatinine, urea and alanine aminotransferase (ALT), Biuret’s colorimetric test (Bradford Diagnostics, Sigma-Aldrich, St. Louis, MO, US) for total proteins, colorimetric methods (Manual Rx Monza (AP542 and CA590), Randox Laboratories Limited, Crumlin, UK) for alkaline phosphatase (ALP), and ion-selective electrode (Microlyte 3+2 ion selective analyser, Kone Instruments, Espoo, Finland) for potassium. The insulin concentration was determined using chemiluminescence immunoassay (Immulite, Siemens Healthcare S.L.U., Madrid, Spain). Urinalysis to measure urine specific gravity was evaluated with a refractometer (Atago T2-NE, Atago Co., LTD, Tokyo, Japan); the urine protein to creatinine ratio (UPC) for proteinuria evaluation was analysed using protein spectrophotometry (DU-20, Beckman Coulter Inc., Brea, CA, US); and reflexion spectrophotometry was employed for creatinine (Refrovet Plus, Scil animal care company, Viernhheim, Germany). The UPC was evaluated when the urinary sediment was considered inactive and a cut-off of 0.5 was established as being positive. The systolic blood pressure was measured using the Doppler ultrasonography method (Vettex Uni900, Huntleigh Diagnostics Ltd., Cardiff, UK) and hypertension was considered when the systolic blood pressure was higher than 160 mmHg. The body condition score was evaluated according to the World Small Animal Veterinary Association nutritional assessment guidelines (Freeman *et al.* 2011). Any adverse events that occurred during the follow-up on the medically treated dogs from the two groups were categorised according to the Veterinary Cooperative Oncology Group criteria (version 1.1) (Anonymous 2016), including: grade 1 (mild), grade 2 (moderate), grade 3 (severe), grade 4 (life-threatening) and grade 5 (death related to adverse event).

**References**

**Anonymous.** Veterinary cooperative oncology group - common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1. *Veterinary and Comparative Oncology* 14, 417–46, 2016

**Freeman L, Becvarova I, Cave N, MacKay C, Nguyen P, Rama B, Takashima G, Tiffin R, Tsjimoto H, van Beukelen P.** WSAVA nutritional assessment guidelines. *Journal of Feline Medicine and Surgery* 13, 516–25, 2011

Supplementary Figure 1. Inclusion flowchart showing canine cases diagnosed with suspect insulinoma, treated surgically or not depending on clinical staging, and then medically treated with palliative therapy alone (CG) or in combination with toceranib (TG) from recurrence after surgery or from diagnosis.

Diagrama

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