DIAGNOSTIC VIABILITY OF CANINE MAST CELL TUMOUR TISSUE BIOPSY SAMPLES ACQUIRED 5 – 10 MINUTES POST-TIGILANOL TIGLATE TREATMENT

Pamela D. Jones[1], Graham K. Brown[1], Kenneth Day[2], William Hendricks[2], Sharadha Sakthikumar[2], Derick Whitley[2]

[1]QBiotics Group Limited, [2]Vidium Animal Health

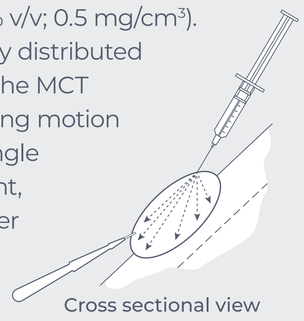
INTRODUCTION

Tigilanol tiglate (TT), a small molecule derived from the *Fontainea picrosperma* (blushwood) plant, is an FDA- and APVMA-approved, intratumoural injection indicated for treatment of canine cutaneous mast cell tumours and subcutaneous mast cell tumours located at or below the elbow and hock.

The majority of canine mast cell tumour (cMCT) patients are diagnosed via fine needle aspiration (FNA) and cytology, procedures which often eliminate the need for sedation or anaesthesia typically required for diagnostic biopsy. This study aimed to explore the feasibility of procuring viable tissue samples for histopathology, genomic analysis, and prognostic evaluation within 5 minutes post-intratumoural tigilanol tiglate when a patient may already be sedated for treatment. This study also evaluated if the biopsy collection affected the efficacy of TT.

MATERIALS AND METHODS

Target lesions in this study were diagnosed and graded cytologically using the Camus system. TT treatment was administered in accordance with the APVMA-approved label protocol. Tumour volume was calculated using a modified ellipsoid method (volume = 0.5 x length (cm) x width (cm) x depth (cm)) determining the appropriate TT dose (50% v/v; 0.5 mg/cm^3). TT was evenly distributed throughout the MCT using a fanning motion through a single injection point, utilising a Luer lock syringe.

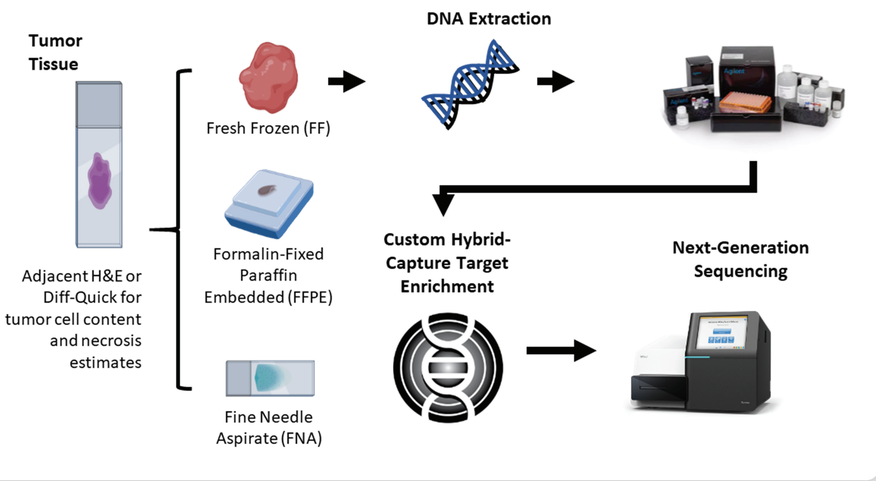


Cross sectional view

Each MCT was biopsied with a 2 mm punch biopsy five minutes post-treatment. Nine cMCT tissue samples were submitted for histopathologic assessment and seven were analysed genotypically using the SearchLight DNA® panel.

Treatment efficacy was primarily assessed through Complete Response (CR), defined as total tumour resolution at Day 28 post-treatment, following modified RECIST criteria from the Veterinary Cooperative Oncology Group. Long-term efficacy was reassessed at 84 days and 12 months post-treatment.

Figure 1. SearchLight DNA™ Workflow and Content. SearchLight DNA™ is a tumour-only, next generation sequencing (NGS), hybrid-capture, canine gene panel covering 482,000 base pairs of 120 genes associated with canine or human cancer. SearchLight DNA™ wet laboratory workflow for sequencing tumour tissue (FF, FFPE, FNA)



RESULTS

The study found that 89% (8 out of 9) of MCTs had a complete response to a single injection. This response rate increased to 100% with a second injection. Figure 3 illustrates the healing process after a single TT injection and post-treatment biopsy. By the 12-month follow-up, only one patient had experienced recurrence. All biopsy samples post-TT injection remained viable with no sample deterioration reported by the pathologist. Although DNA yield was low (<50 ng) in 4/7 samples, mutations were detected in cMCT-associated genes including KIT, PTEN, and SETD2. Novel mutations were also discovered in BRAF, CDK4, and MEN1 genes.

Figure 2. Pre-treatment cytology of a low-grade mast cell tumour (A). Neoplastic mast cells have histologically well-preserved cellular features with no morphologic changes associated with treatment (B).

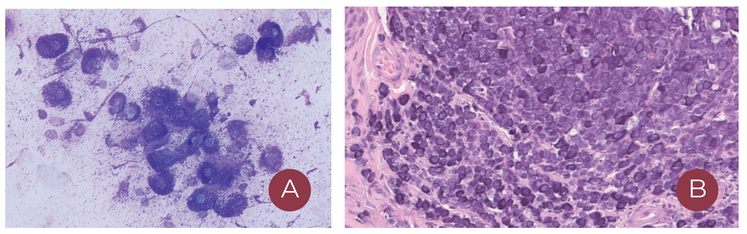


Figure 3. Study patient was assessed before treatment (A), the MCT (volume = 4.3 cm^3) received a single TT treatment and was biopsied (arrow) on Day 0 (B), progress monitored on Days 1 (C), 7 (D), and 14 (E). A complete response was recorded on Day 29 (F). No recurrence detected 12 months post-treatment

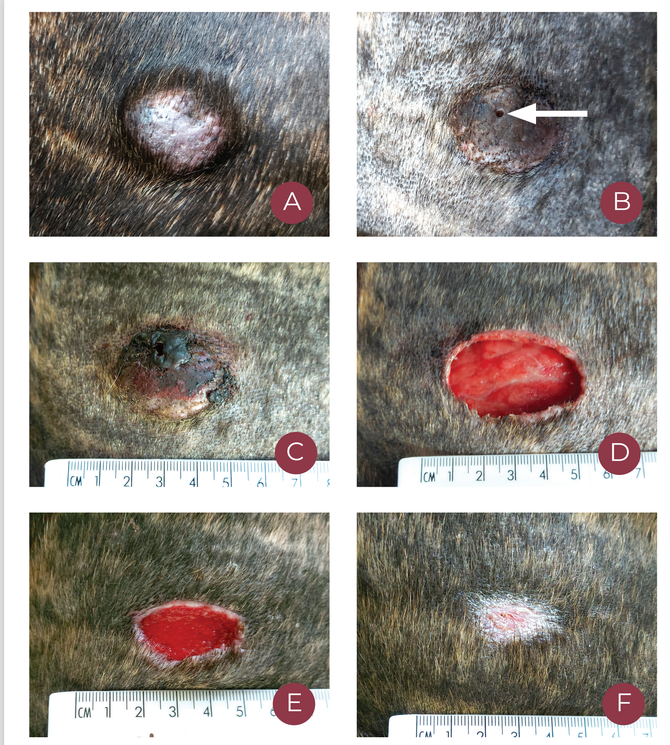
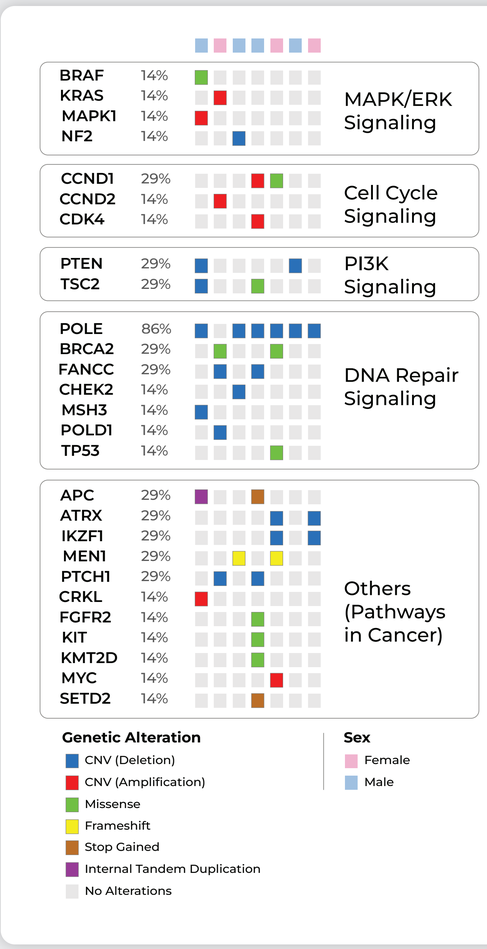


Table 1. Summary demographics for the 8 dogs in the study

	C25, N = 8
Patient age (years)	8 (4,11)
Sex	
Female	5 (62%)
Male	3 (38%)
Weight (Kg)	30 (16,45)
Number of MCT treated	9
Location	
Head	0 (0%)
Trunk	3 (33%)
Upper limb	4 (44%)
Lower limb	2 (22%)
Perineal	0 (0%)
MCT volume (cm^3)	1.5 (0.0,7.7)

Figure 4. Oncoprint showing genes bearing mutations in 7 sequenced patient samples



CONCLUSIONS

The results suggest that post-tigilanol tiglate biopsy provides viable tissue samples for histopathological and genomic diagnostics without compromising treatment efficacy. A larger tissue sample could potentially enhance confidence in assigning mitotic index and histologic grade and may improve DNA yield and quality for genomic evaluation.

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CONFLICT OF INTEREST STATEMENT

Pamela D. Jones and Graham K. Brown are employed by QBiotics Group Limited. Kenneth Day, William Hendricks, Sharadha Sakthikumar and Derick Whitley are employed by Vidium Animal Health. QBiotics Group Limited own the intellectual property, marketing authorisation and patents associated with tigilanol tiglate. SearchLight DNA® is a product developed and provided by Vidium Animal Health.

QBiotics Group