Genomic Profiling Reveals Aggressive Molecular Features in a Histologically Indolent Canine T-Cell Lymphoma, Consistent with Clinical Behavior

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Case Background

Kubo, an 8-year-old German Shepherd, presented with mild generalized lymphadenopathy. Flow cytometry (CD3+, CD45+, high proliferative index) and early chemosensitivity supported an aggressive T-cell phenotype, whereas lymph-node histopathology favored an indolent variant. This created a biologic discordance suggestive of composite/transforming disease, sampling bias, or even an occult (aleukemic) leukemic process not readily appreciable in the node.

To resolve lineage and assess risk in alignment with tumor biology, and to interrogate therapeutic and prognostic biomarkers, VetOmics Canine Comprehensive Genomic Profiling (Canine CGP) was performed, with plans for molecular response assessment and bone marrow evaluation when feasible.

Genomic Findings and Interpretation

Canine CGP performed on Kubo's lymph node FNA identified 8,297 mutations across 7,928 genes, in a specimen of estimated 70% tumor purity. Two well-established activating mutations act as main putative driver mutations, *NRAS* G13V (41% variant allele frequency) and an *FLT3* exon 14 insertion (E579_Y580insATLIFREYE at 19% VAF).

FLT3 alterations are primarily described in human hematologic malignancies, but they have also been reported in canine acute lymphocytic leukemia and mast cell tumors, and within VetOmics cohort (PMID 21272320; PMID 36072760). *NRAS* mutations are also well documented across many human and canine cancers.

FLT3, a class III receptor tyrosine kinase, when activated by activating point mutations, internal tandem duplications, rearrangements, or exon-14 insertions, leads to constitutive MAPK, PI3K/AKT, and JAK/STAT signaling, promoting unchecked proliferation and survival. NRAS, a small GTPase that operates downstream of RTKs (including FLT3), drives persistent MAPK signaling when mutated at canonical hotspots such as G13.

Together, these alterations reinforce oncogenic signaling and are associated with aggressive disease biology, treatment resistance, and poor prognosis in human hematologic malignancies, including AML, B-cell ALL, myelodysplastic syndrome (MDS), and myelofibrosis (PMID: 12970773; 8329714; NCCN.org).

The lower allele frequency of the FLT3 mutation relative to the NRAS suggests that the FLT3-mutant clone may have arisen later during tumor evolution, or represents a minor subclone contributing to progression. These findings raise the possibility that this case represents a molecular transition from an early-stage indolent lymphoma to a more aggressive form, which may explain the discrepancy between the histopathologic and clinical behavior. We have observed similar scenarios in other T-cell lymphoma cases, and increasingly we are able to delineate the underlying mechanisms through comprehensive genomic profiling.

In this context, Kubo's mild interval progression, along with repeat flow cytometry showing a substantial CD34 positive, lineage poor blast population, suggests that the primary process may have been an occult acute leukemia from the outset rather than an indolent nodal lymphoma with secondary transformation. The lack of baseline bone marrow sampling limited recognition at diagnosis.

Precision medicine in action: Diagnostic clarification and Genomic-Guided Treatment Recommendation

Canine CGP resolved the key ambiguity by revealing an NRAS G13V founder lesion with a subclonal FLT3 exon-14 insertion population, an axis that coherently explains the discordance between histology and clinical behavior and supports an NRAS/FLT3-driven, biologically aggressive process with capacity for clonal evolution (up to a leukemic/blastic phase). The genomic context refines classification and provides clinically actionable biomarkers, while enabling assessment of disease dynamics and response tracking through allele specific NRAS and FLT3 VAF assessment.

Therapeutic recommendations prioritized mutation/pathway-driven targets, with the strongest rationale in this context.

- **Sorafenib:** multikinase inhibitor targeting FLT3 and MAPK signaling
- **Trametinib:** MEK inhibitor, targets RAS/MAPK pathway
- **Midostaurin + cytarabine:** chemotherapy plus a broader FLT3 inhibition for leukemic/blastic phase
- **Gilteritinib:** selective FLT3 inhibitor for relapsed/refractory disease or leukemic phenotype

These approaches are pursued within a trial or compassionate framework with rigorous safety monitoring (CBC and chemistry, hepatic profile, blood pressure and urinalysis) and predefined response metrics (flow cytometry, imaging), alongside supportive care to maintain quality of life.

Why This Matters

Kubo's case demonstrates how high-resolution genomic profiling can reconcile discordant histology and phenotype in T cell disease, where morphology alone may under call biological risk or miss aleukemic leukemic phases. Defining the NRAS and FLT3 axis aligns classification with clinical behavior, refines prognosis, and anticipates clonal evolution. Integrating Canine CGP with pathology and flow cytometry enables risk adapted, mechanism based therapy and objective response surveillance.

This genomics-guided precision framework complements histopathology and other diagnostic methods, converting a deeper understanding of tumor biology into patient-specific, actionable decisions.