Viral clonal landscape and tumor progression: lessons from tumor-resistant sheep in the BLV leukemia model

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Background:
Bovine Leukemia Virus (BLV), a deltaretrovirus closely related to HTLV-1, induces B-cell leukemia/lymphoma in ~5% of infected individuals (cattle) after a protracted asymptomatic infection. While not a natural host, experimentally-infected sheep systematically develop leukemia after a shorter asymptomatic period, providing a unique model for studying tumor progression *in vivo*. Unlike HTLV-1, BLV produces a cluster of highly-conserved abundantly-expressed miRNAs. Here, we exploit the opportunity of being able to screen an unusual cohort of sheep infected with miRNA-impaired BLV variants which we found resistant to tumor development, and investigate the impact of the viral clonal landscape on tumor progression.

Methods:
We have accumulated longitudinal samples from wild-type and miRNA-altered BLV-infected sheep over >9 years (>75 time-points), generating a unique bio-bank of primary cells covering all stages of the disease. Using this source of genetic material we explored provirus insertional signatures and clonal landscapes by NGS clonality.

Results:
Although deleting or inverting BLV miRNAs did not prevent the virus from infecting sheep, this dramatically impacted disease evolution. All wild-type-infected animals developed leukemia following a consistent/gradual increase in proviral load (PVL). In contrast, not a single animal infected with miRNA-impaired variants developed malignancy. These animals were characterized by extremely low and stable PVLs. We observed dramatic changes in the animals' clonal landscapes, as indicated by the loss of a strong hotspot signature, the absence of a significant orientation bias of closely-located proviruses and a scattered distribution of proviral integration sites. More intriguingly, the tumor-resistant cohort showed an atypical mono/oligoclonal-like clonal distribution with dominant clones characterized by increased survival while such signatures were not observed in wild-type-infected asymptomatic animals at any PVL.

Conclusion:
Altogether, observations in a long-term surviving sheep cohort will contribute to our understanding of mechanisms by which a given clonal landscape prepares a suitable terrain favouring progression towards uncontrolled expansion.

Disclosure of Interest Statement:
Nothing to disclose.