Frederick National Laboratory for Cancer Research

Xenograft-associated B cell lymphoproliferative disease as a surrogate model to study Epstein-Barr Virus (EBV) driven lymphoma of the elderly

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Abstract

Background: Patient-derived tumor xenografts (PDX) are powerful tools to study cancer biology, cancer genomics and developmental therapeutics. A common problem in the development of PDX models is proliferation of atypical lymphocytes at the implant site, which often overtake or limit the growth of the original tumor. This atypical proliferation has been described as Xenograft-Associated B cell Lymphoproliferative Disease (XABLD) in our PDX models. In this study, we characterized XABLD cases by morphology, immunophenotyping and genomic profiling. We hypothesize that XABLD tumors are morphologically and phenotypically similar to EBV-driven lymphoma of the elderly and may function as a surrogate model for that lymphoma.

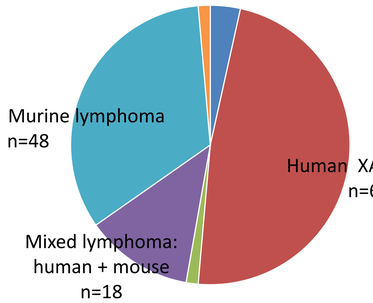
Materials and Methods: Models were generated from patient tissue collected under NCI Tissue Procurement Protocol (clinicialtrials.gov: NCT00900198) and CIRB Tissue Procurement Protocol 9846 for development of models for NCI's Patient-Derived Models Repository (https://pdmr.cancer.gov). Specimens were implanted subcutaneously in NOD/SCID/IL2Rg null (NSG) mice and animal health was monitored throughout the study. Tumors in mice with suspected XABLD were harvested and reviewed by histology and immunohistochemical analysis for CD45, B and T cell markers and EBV status. All samples in this study were classified by the Lymph2Cx NanoString cell of origin assay and transcriptome profiling.

Results: XABLD-associated mice had rapidly growing CD45-positive tumors at the implantation site. Histopathological features were consistent with EBV-driven diffuse large B-cell lymphoma (DLBCL) primarily of polymorphous subtype. All XABLD specimens were diffusely positive for CD20 and EBNA, and most cases contained tumor infiltrating CD8-positive T-cells. Out of 42 cases, 36 were PD-L1-positive and 26 were PD-1-positive by IHC. 39 cases exhibited an activated B cell (ABC) phenotype, which is predominant in EBV-positive DLBCL.

Conclusion: XABLD development has been seen across multiple patient histologies from both solid tumor and circulating tumor cells tissues of origin. The clinical presentation, morphology and molecular characteristics of XABLD cases were similar to EBV-driven DLBCL. As DLBCL is an aggressive disease with limited treatment options, our early-passage XABLD models may be useful in the preclinical evaluation of new therapies for EBV-positive DLBCL.

XABLD prevalence in the NCI Patient-Derived Model Repository (PDMR)

Distribution of lymphoproliferative and autoimmune cases in mice (n=144)

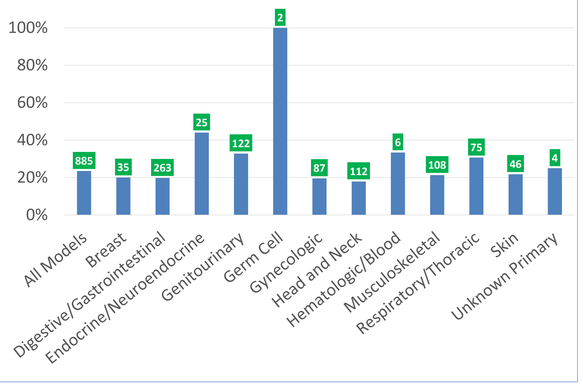


Lymphoproliferative disease occurrence by passage:
- P0: 84%
- P1: 10%
- P2: 5%
- P3: 1%

Sample type	XABLD	PDX model
CTC	8	7
biopsy/resection	61	248

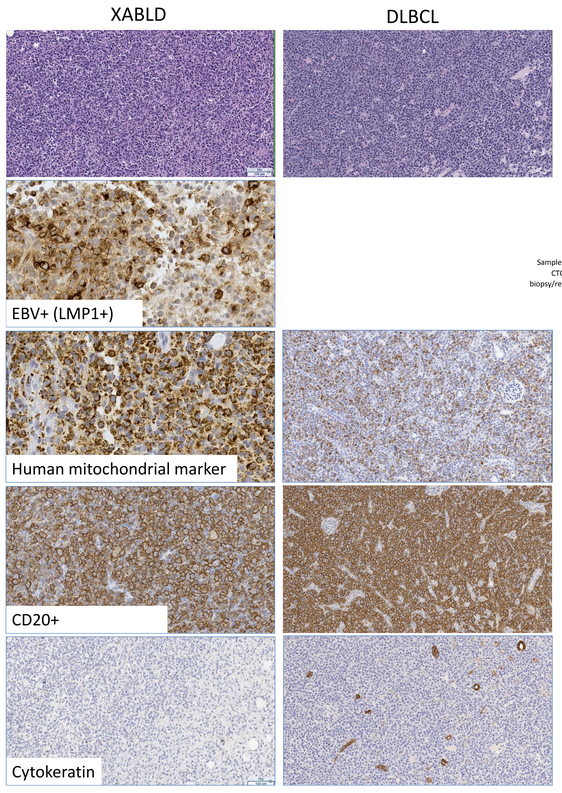
- GvHD (n=5)
- Mixed Dx in patient: solid tumor + lymphoma (n=2)
- B cell lymphoma Dx in patient (n=2)

XABLD frequency by histology

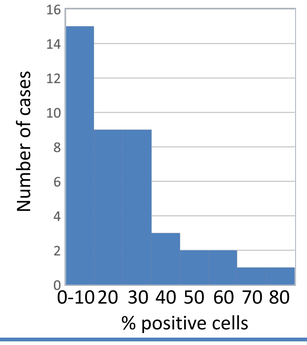


XABLD exhibit features of DLBCL-like B cell lymphoma

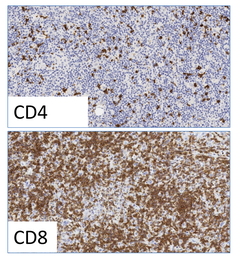
- 42 characterized cases
- EBV+, human mitochondrial marker+, CD45+
- Morphological diagnosis:
  - Polymorphic: 33
  - Monomorphic: 9
- CD20 IHC: strong positive
- Lymph2Cx phenotype classification:
  - Activated B cell (ABC): 39
  - Germinal B cell (GCB): 2
  - Unclassified: 1
- PD-L1 IHC: mainly cases with low fraction of PD-L1 positive cells



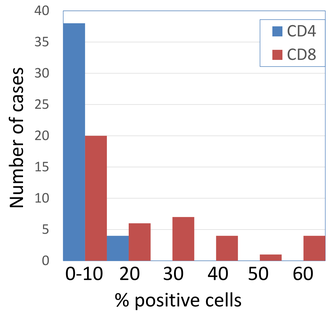
Frequency of PD-L1+ lymphoma cells



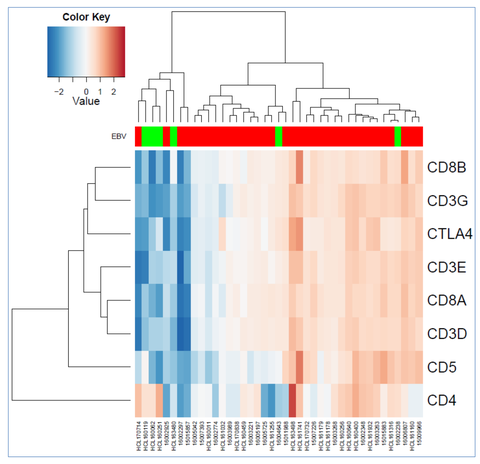
Some XABLD cases have significant T cell involvement



Frequency of CD4 and CD8 T cells in XABLD (passage 0)

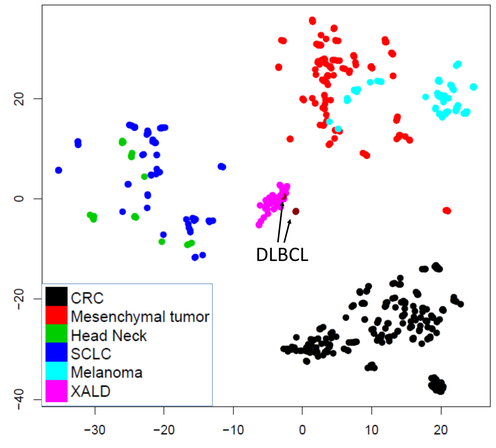


- mRNA expression: T cell markers (NanoString)



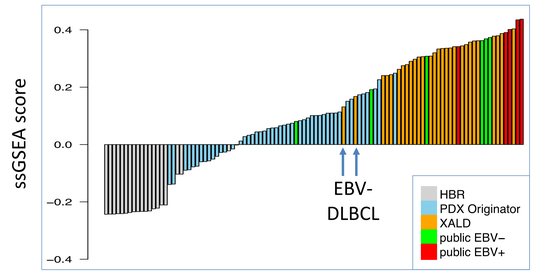
XABLD cases cluster with ABC-subtype DLBCL

- t-SNE plot: RNA-seq data of XABLD cases (n=26)



- Gene expression clustering of XABLD, DLBCL and selected solid tumor histologies from the public PDMR collection
- Additional EBV+ DLBCL gene expression datasets will be obtained to confirm that XABLD transcriptomes are similar to EBV+ DLBCL

XABLD models exhibit elevated NF-kB pathway activity



- EBV+ DLBCL are known to have elevated NF-kB signaling
- Single-sample GSEA enrichment scores for 24 NF-kB target genes
- Public EBV- and EBV+: Published DLBCL data[1]

Conclusions

- XABLD are lymphomas originating from B cells present in solid tumors
- XABLD represent EBV positive DLBCL-like tumors
  - EBV-positive
  - ABC phenotype
  - NF-kB activation signature
  - Transcriptome profile may be similar to DLBCL
- Some XABLD models have significant T cell involvement
- XABLD may be useful as surrogate DLBCL models for preclinical research

Future work

- Further characterized XABLD for:
  - IGH and IGK B-cell clonality assay
  - EBV latency typing
- Compare gene expression profile of XABLD to DLBCL
- Characterize mutations and aneuploidy by whole exome sequencing
- Generate XABLD cell line models
- Compare treatment response of XABLD and DLBCL models

Acknowledgments

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References

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