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 implantation site, which often overtake or limit the growth of the original tumor. This atypical lymphocyte 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 post-transplant lymphoproliferative disease (PTLD) and diffuse large B cell lymphoma (DLBCL). XABLD is a surrogate model to study EBV-driven PTLD and DLBCL.

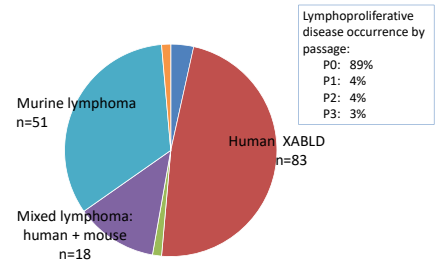
Materials and Methods: Models were generated from patient tissue collected under NCI Tissue Procurement Protocol (clinicaltrials.gov: NCT00900198) and CIRB Tissue Procurement Protocol 9846 for development of models for NCI's Patient-Derived Models Repository (https://pdmr.cancer.gov). Specimens were 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, EBV status, B-cell clonality assay. All samples were also classified by the Lymph2Cx NanoString cell of origin assay and transcriptome profiling.

Results: XABLD cases were found to originate from both solid tumor and circulating tumor cell implants. XABLD is a rapidly growing tumor positive for CD45, CD20, and LMP1 stains, 36 of 42 cases are strongly positive for PD-L1 stain. 39 of 42 cases exhibited an activated B cell (ABC) phenotype with evidence of elevated NF-kB signaling. Most cases were monoclonal for IGK/IGH and contained high numbers of tumor infiltrating CD8-positive T-cells with associated high mRNA expression of activated T cell markers.

Conclusions: The clinical presentation, morphology and molecular characteristics of XABLD cases were similar to EBV-driven DLBCL. As the XABLD models exhibited frequent PD-L1 expression and marked infiltration of CD8-positive T cells, they may be useful for in vitro evaluation of checkpoint inhibitor response and T cell antitumor activity.

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

Distribution of lymphoproliferative and autoimmune cases (n=161)

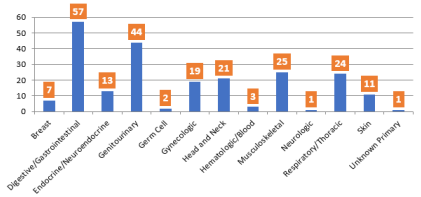


Lymphoproliferative disease occurrence by passage:
P0: 89%
P1: 4%
P2: 4%
P3: 3%

Sample type	XABLD	PDX model
CTC	10	7
biopsy/resection	73	248

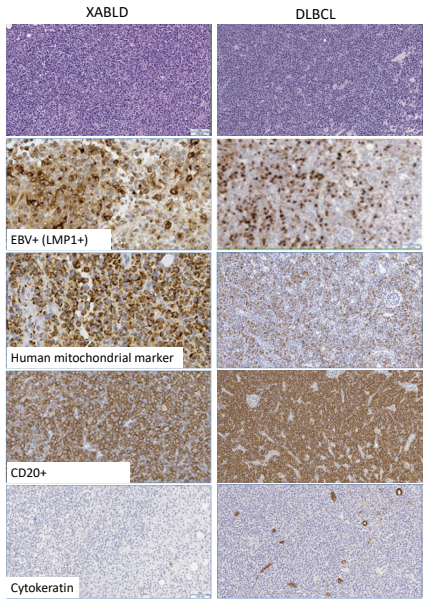
- GvHD (n=5)
- Mixed Dx in patient: solid tumor + lymphoma (n=2)
- B cell lymphoma Dx in patient (n=2)

In Vivo PDX Models - XALD/Human Lymphoma Status by PDM Assigned Body Location

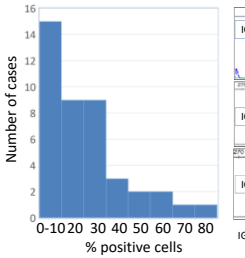


XABLD exhibit features of DLBCL-like B cell lymphoma

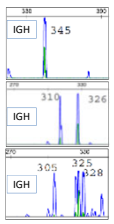
- 42 characterized cases
- EBV+, human mitochondrial marker+, CD45+
- Morphological diagnosis:
  - Polymorphic: 33
  - Monomorphic: 9
- CD20 IHC: strong positive
- Lymph2Cx phenotype classification:
  - Activated B cell (ABC): 39
  - Germinal B cell (GCB): 2
  - Unclassified: 1
- PD-L1 IHC: mainly cases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



Frequency of PD-L1+ lymphoma cells



XABLD cases are monoclonal or biclonal



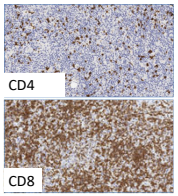
Monoclonal: 28 of 44

Bi-clonal: 12 of 44

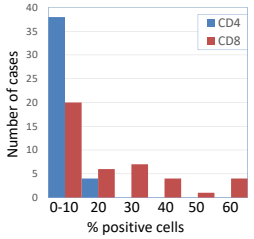
Oligoclonal: 4 of 44

IGH + IGK B cell clonality assay (Invivoscribe)

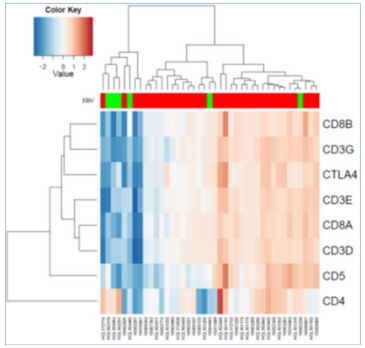
Some XABLD cases have significant T cell involvement



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

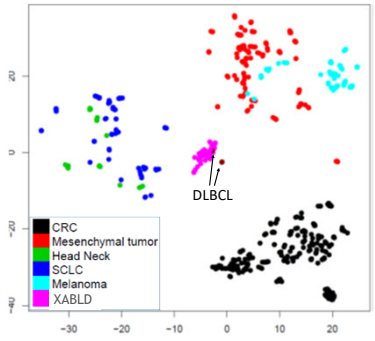


➢mRNA expression: T cell markers (NanoString)



XABLD cases cluster with ABC-subtype DLBCL

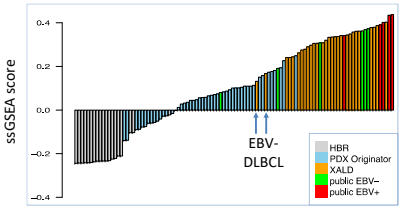
➢t-SNE plot: RNA-seq data of XABLD cases (n=26)



➢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

References

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