Modeling R-CHOP chemoimmunotherapy in murine lymphoma to characterize and overcome resistance

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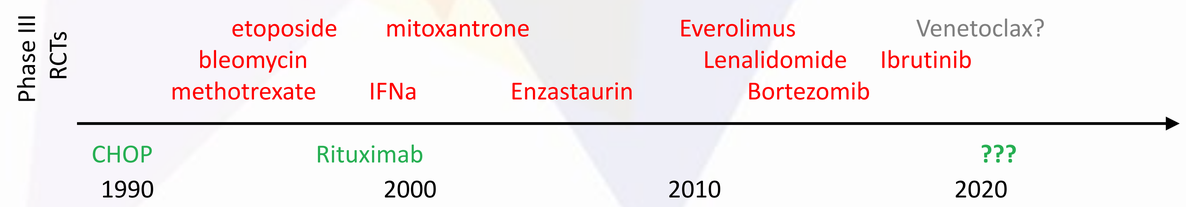
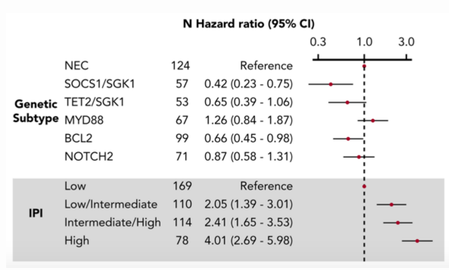
Abstract

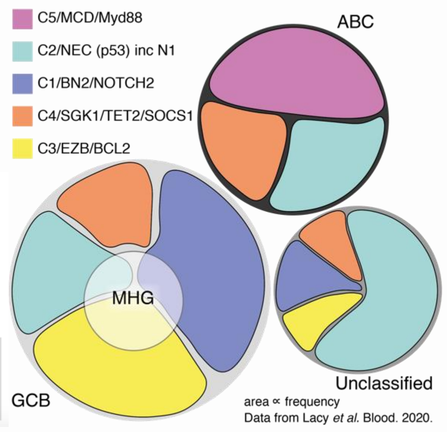
First line therapy for diffuse large B cell lymphoma (DLBCL) has not improved for 20 years, despite numerous randomized trials. Rational development of new R-CHOP combinations requires a more sophisticated model for *in vivo* testing, which captures both the heterogeneity of newly diagnosed DLBCL and the role of the immune system in mediating the response to therapy. We have developed a spontaneous immunocompetent mouse model of DLBCL, of the activated B cell (ABC) subtype, and studied the surviving lymphoma cells by RNAseq after treatment with murine anti-CD20 plus CHOP. Mice with B cell-specific overexpression of MYC, IKK2 (inhibitor of kappaB kinase beta) and deletion of Prdm1 develop a high- grade large B cell lymphoma after 6-8 months which recapitulates the features of human ABC lymphoma: widespread lymphadenopathy with frequent extranodal manifestations, expression of IRF4 but not BCL6, and high proliferative index by Ki67 staining. Treatment with 5D2 (anti-CD20), CHOP, or the combination effectively shrinks lymphoma tissues, and the population of surviving cells demonstrate transcriptional changes which may identify pathways for therapeutic targeting in combination with R-CHOP.

Unmet needs in DLBCL

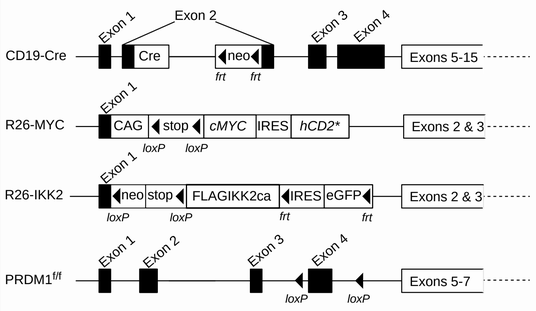


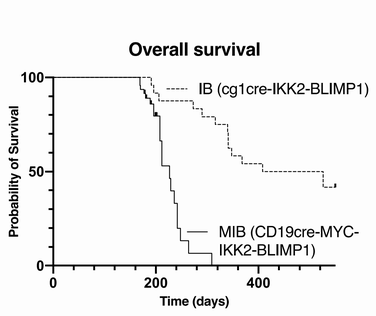
- Although CAR T cells represent a major advance in relapsed DLBCL, 20 years of randomised trials have not improved on R-CHOP first line.
- The extensive biological heterogeneity of DLBCL may have prevented these targeted agents demonstrating efficacy in all comers.
  The MCD/C5/Myd88 molecular subgroup is the only group in the new classicifications associated with an adverse prognosis independent of IPI.

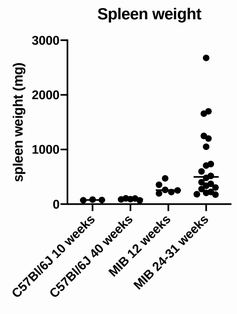




The MIB mouse: a tractable model of ABC DLBCL

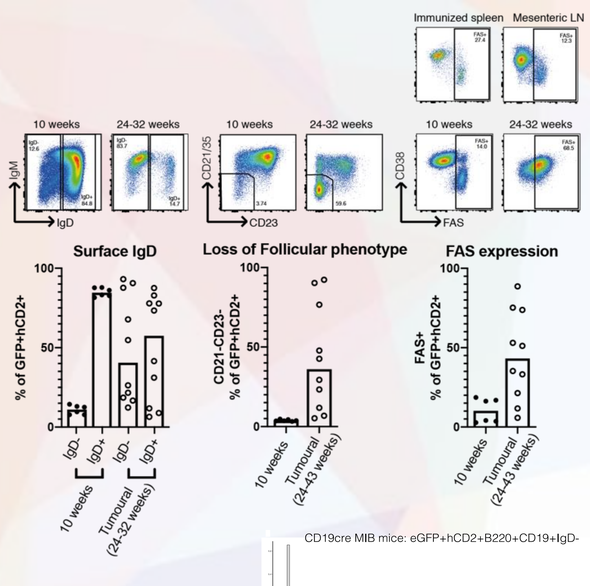


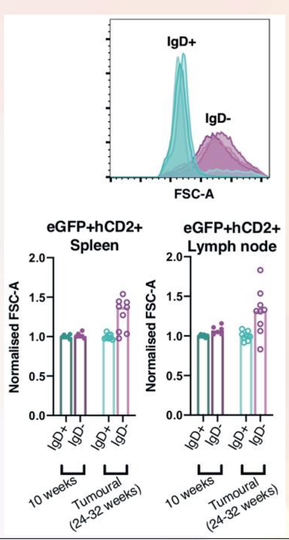


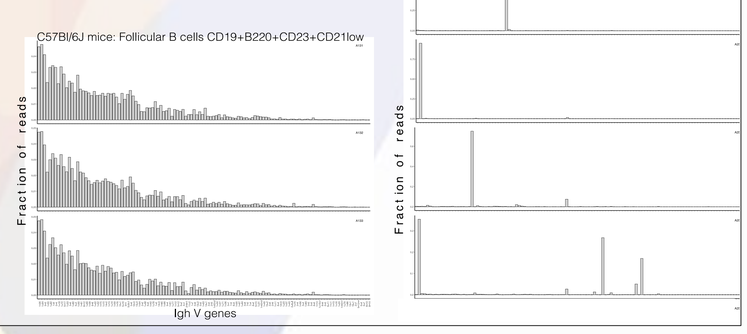


- Murine DLBCL of the ABC subtype has previously been described after conditional expression of IKK2 and deletion of BLIMP1, using cre-lox technology. (Calado et al. 2010) This activates NF-kB signaling and prevents plasma cell differentiation. These are characteristic of the MCD/C5 subtype.
- The latency period of this model is too long for therapeutic studies. We added conditional MYC expression to accelerate lymphomagenesis.
- Using a CD19-cre construct to drive recombination in B cells we observed lymphoma development in 6-7 months, associated with marked lymphadenopathy, splenomegaly and extranodal involvement.

- Despite cre-mediated recombination occurring early in B cell development, we observed a mature antigen-experienced phenotype in lymphoma cells which was not present in recombined B cells of young mice, prior to lymphoma development.
- High FSC-A of recombined cells was selectively present in IgD- cells at lymphoma development.
- Transcriptional analysis of sorted lymphoma cells (eGFP+hCD2+CD19+B220+IgD-) revealed clonal expression of a single IgH V region.

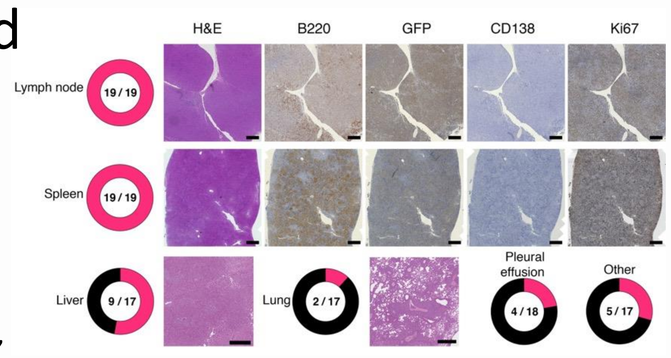




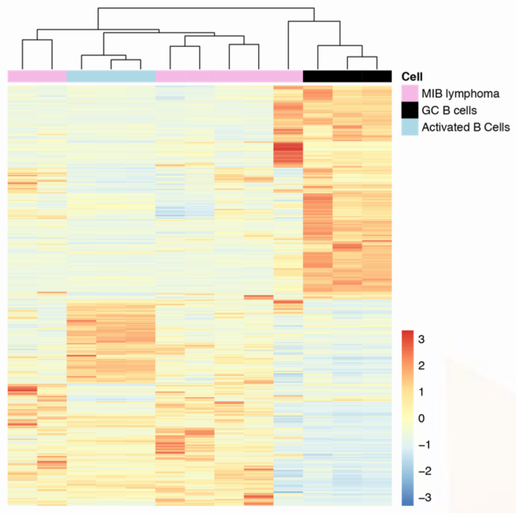


The MIB mouse cont'd

- The histology and immunophenotype of lymphomas of MIB mice is consistent with ABC type DLBCL, with some heterogeneity in, for example CD5 and Fas expression.
- Supervised clustering using those genes differentially expressed in germinal centre B cells demonstrates that in 6 of 7 samples, lymphomas clusters with ABC samples.

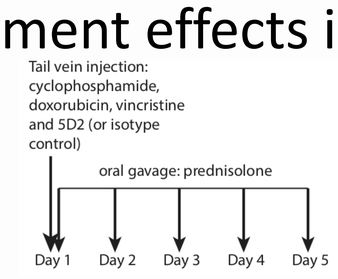


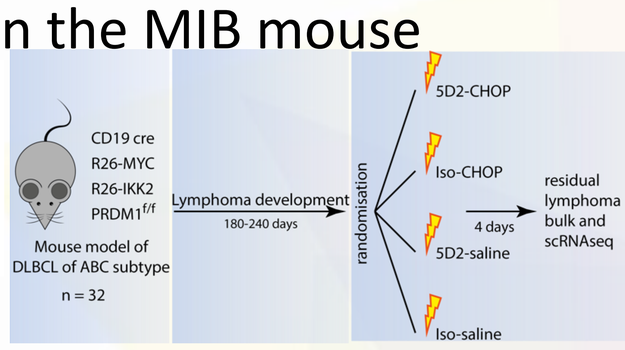
Mouse	Tissue	Dominant population	CD19	B220	Isotype	CD38	CD21	CD23	Fas	CD138	CD5	CD93
1	LN	GFP+hCD2+	+	weak	IgD– IgM+	+	–	–	weak	–	+	–
	spleen	GFP+hCD2+	+	+	IgD+ IgM+	+	+	+	–	–	–	–
2	LN	GFP+hCD2+	+	+	IgD+ IgM+	+	+	+	weak	–	–	–
	spleen	GFP+hCD2+	+	+	IgD+ IgM+	+	+	+/–	–	–	–	–
3	LN	GFP+hCD2+	+	+	IgD– IgM+	+	–	–	weak	–	+	–
	spleen	GFP+hCD2+	+	+	IgD+/– IgM+	+	–	–	–	–	–	–
4	LN	GFP+hCD2weak	+	+	IgD+ IgM+	+	+	+	–	–	–	–
	spleen	GFP+hCD2weak	+	+	IgD+/– IgM+/–	+	+/–	+/–	–	weak	–	–
5	LN											
	spleen	GFP+hCD2+	+	+	IgD– IgM+	+	–	–	weak	–	–	–
6	LN	GFP+hCD2+	+	+	IgD+ IgM+/–	+	–	+/–	weak	–	–	–
	spleen											
7	LN	GFP+hCD2+	+	+	IgD+/– IgM+	+	–	–	weak	–	–	–
	spleen	GFP+hCD2+	+	+	IgD– IgM+	+	–	–	weak	–	–	–
8	LN	GFP+hCD2+	+	+	IgD+/– IgM+	+	+/–	–	–	–	–	–
	spleen	GFP+hCD2+	+	+	IgD+ IgM+	+	+	+	–	–	–	–
9	LN	GFP+hCD2+	+	+	IgD+IgM–	+	+/–	+/–	–	–	+/–	–
	spleen	GFP+hCD2+	+	+	IgD– IgM+/–	+	–	–	–	–	–	–
10	LN	GFP+hCD2+	+	+	IgD+ IgM+/–	+	+	+	weak	–	+/–	–
	spleen	GFP+hCD2+	+	NA	NA	+	–	–	–	NA		–
11	LN											
	spleen	GFP+hCD2+	+	+	IgD– IgM+	+	–	–	weak	–	–	–
12	LN	hCD2+	+	+	IgD– IgM–	+	–	–	weak	weak	+	–
	spleen	hCD2+	+	+	IgD– IgM–	+	–	–	weak	weak	+	–



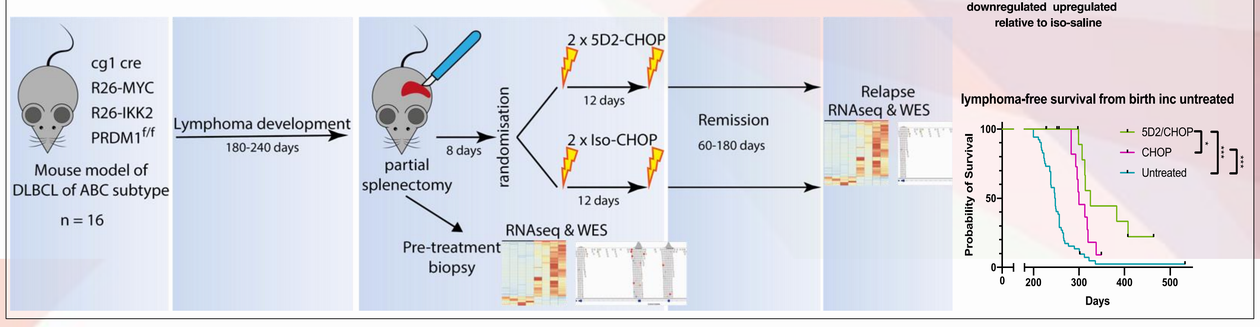
Post-treatment effects in the MIB mouse

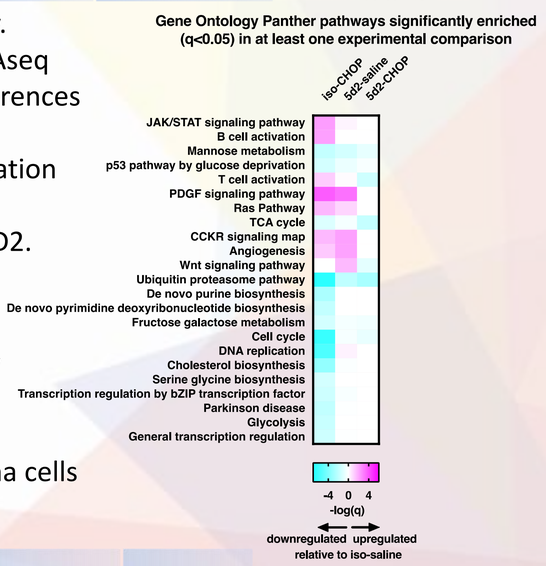
- The murine type 1 anti-CD20 antibody 5D2 was combined with CHOP chemotherapy to model human treatment.
- 4 cohorts of MIB mice were randomised to 5D2, CHOP, 5D2-CHOP or an isotype control antibody.
- Lymph nodes were harvested four days later and RNAseq performed on sorted lymphoma cells to identify differences present in surviving cells.
- Gene ontology enrichment demonstrates downregulation of replication pathways in cells surviving CHOP, and upregulation of PDGF, Ras and Wnt pathways after 5D2.





- In a parallel experiment, using a cg1-cre construct, mice with lymphoma underwent partial splenectomy prior to 2 cycles of 5D2-CHOP or isotype-CHOP, and then observed until relapse.
- RNAseq and WES was performed on sorted lymphoma cells pre-treatment and post-relapse. Results are awaited.





Conclusions and future plans

- Complex genetic models of lymphoma can be tractable model systems for studying therapeutic responses.
- The combination of NF-kB activation, MYC expression and BLIMP1 deletion generates high grade B cell lymphomas which closely reproduce features of ABC DLBCL such as IgM+, FAS+ and extranodal involvement, particularly characteristic of the MYD/C5/Myd88 molecular subgroup.
- The transcriptional profile of surviving cells after CHOP chemotherapy is consistent with elimination of highly proliferative cells, consistent with the known mechanism of cytotoxic drugs.
- The higher expression of genes in the PDGF, Ras and Wnt signaling pathways after 5D2 treatment leads to the hypothesis that these pathways contribute to survival of cells after 5D2 and may be therapeutic targets.
- We will use CRISPR/Cas9 to target these pathways and assess their effect on responsiveness to 5D2-CHOP and ultimately devise pharmacological strategies to target them *in vivo*.

References and acknowledgements

1. Lacy *et al*. (2020) Targeted sequencing in DLBCL, molecular subtypes, and outcomes: a Haematological Malignancy Research Network report. *Blood* **135**(20):1759-71.
2. Calado *et al.* (2010) Constitutive canonical NF-κB activation cooperates with disruption of BLIMP1 in the pathogenesis of activated B cell-like diffuse large cell lymphoma. *Cancer Cell* **18**(6):580-9.

BM is funded by a CRUK doctoral clinical fellowship (Accelerator Award C422/A26084). Thanks to Peter Johnson, Jessica Okosun and Peter Van Loo for feedback and suggestions. 5D2 antibody kindlyCrick Institute is funded by grants from CRUK, MRC and the Wellcome Trust.