Improving conventional prognosticators in diffuse large B cell lymphoma using marker ratios

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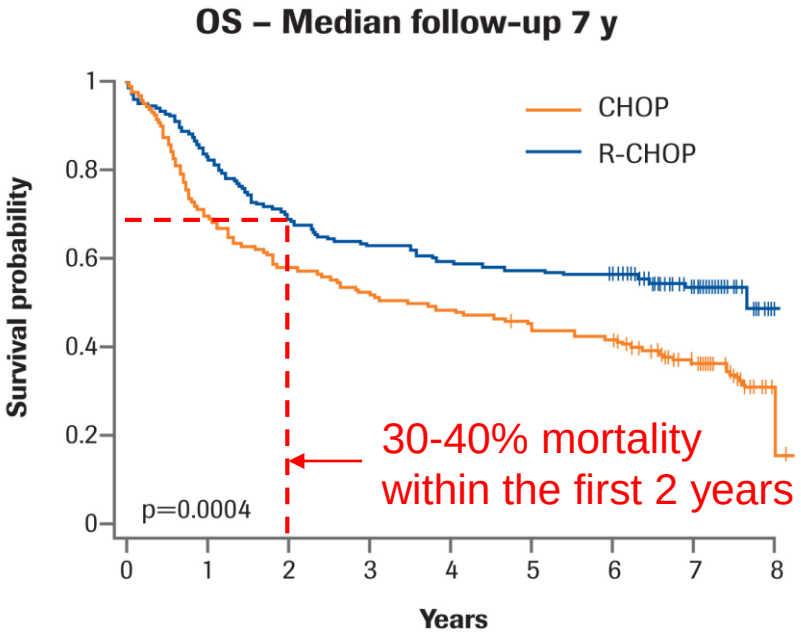
DIFFUSE LARGE B-CELL LYMPHOMA

- Incidence of lymphoma more than doubled in last 20 years
- 6th most common cancer
- Risk by age 85yo of Non Hodgkin's Lymphoma (NHL) is 1 in 42
- DLBCL is most common subtype in adults (30-40% of all cases in Australia) and is the most aggressive form of NHL

Biggest risk factor (by far) is **impaired immunity**

TREATMENTS AND SURVIVAL

Of the majority of patients that die from DLBCL, 30-40% will do so within 2 yrs despite chemotherapy



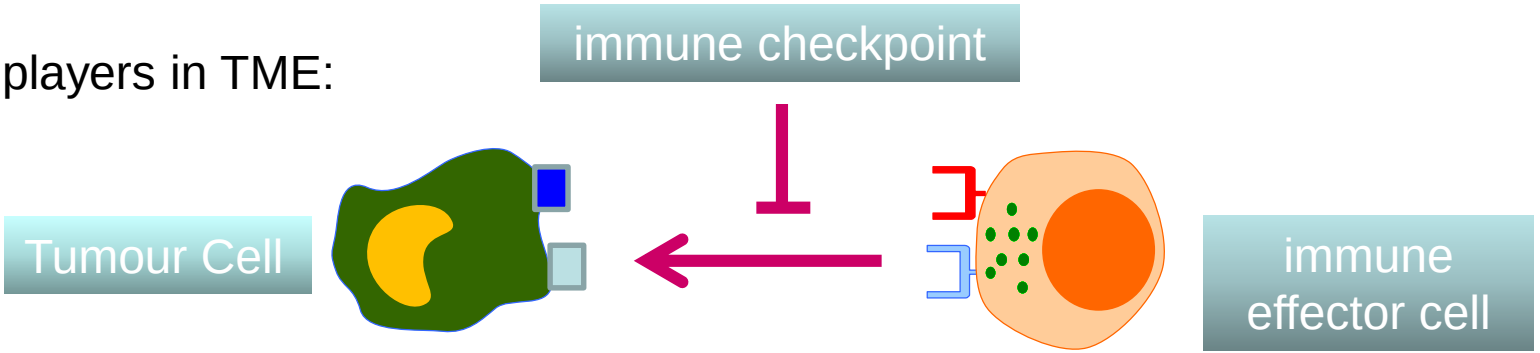
- Addition of **R**ituximab to combination chemotherapy CHOP improves survival in DLBCL by 10-15%
- Exposure to R-CHOP induce chemo-resistance and toxicity in certain group of patients
- Need to more accurately predict response to R-CHOP to **stratify patients** and **identify new immune target agents**

WHY DO PRE TREATMENT PROGNOSTICATORS FAIL?

Cell of Origin (COO), international prognostic index (IPI) and i-PET/CT still fail to identify patients that will relapse

Tumour microenvironment (TME) contributes to tumour initiation, progression and responses to therapy

Three players in TME:



⇨ need to understand **interaction of malignant B cell and anti-tumour immune response** using **gene expression**

IMMUNOMODULATION IN TUMOURS: AN ITALIAN SPAGHETTI FILM

Immune effectors **drive potent anti-tumour responses**

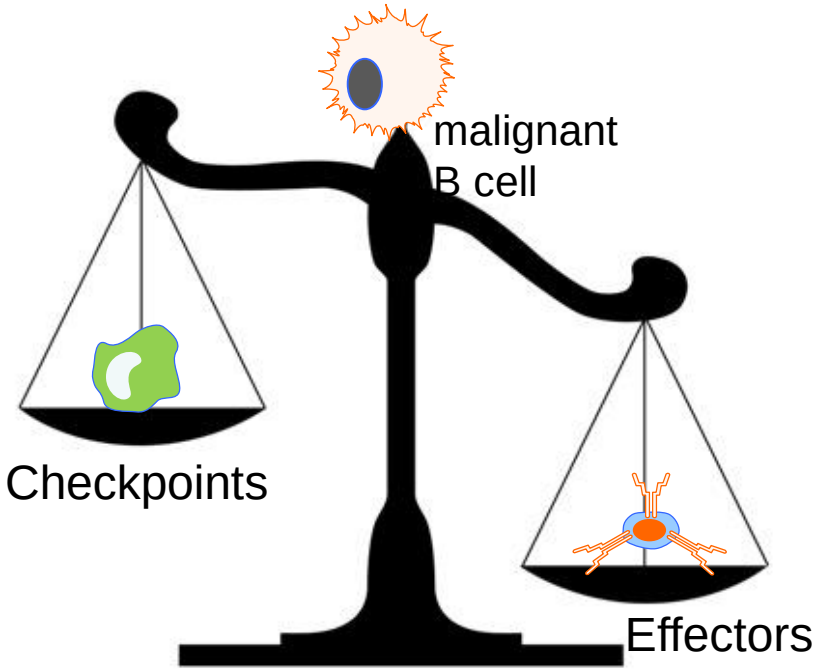
Tumour cells produce **host-checkpoint responses:** dampen immune-effectors, induce **immunosuppressive microenvironment**

In solid-organ cancers, tumor biopsies display ‘adaptive resistance’.

Is it also the case in DLBCL?

Creative idea from Prof. Maher Gandhi

A FINE BALANCE BETWEEN IMMUNE EFFECTORS AND CHECKPOINTS TO PREDICT OUTCOME?



Hypothesis:
The relative composition of antagonist checkpoints and immune effectors is prognostic to DLBCL

Does the **ratio of**
Immune Effector(s) / Checkpoint(s)
predict outcome?

DATA

Nanostring™ digital multiplex gene expression (Discovery data)

- 158 de novo DLBCL treated with R-CHOP from 2 Australian centres (Brisbane, Canberra)
- Formalin-fixed paraffin embedded tissue (FFPET) + survival outcome
- Median follow up: 4 yrs; Median age: 62 (27-86)

Affymetrix gene expression (Validation data)

- 233 patients treated with R-CHOP
- Fresh-frozen samples + survival outcome
- Median follow up: 2.8 yrs;
- Publicly available data (GSE10846*)

*Lenz, G., et al. (2008), *Stromal gene signatures in large-B-cell lymphomas.* New England Journal of Medicine. **359** (22)

GENE EXPRESSION RATIOS

Investigating a fine balance between immune effectors and check points

- Immune-Effectors in **numerator**: CD4, CD8, CD56 and CD137
- Immune-Checkpoints in **denominator**: CD163, CD68, **M2 (CD163/CD68)**, PD1 and **PDL1**

Various combinations of gene expression ratios assessed:

1. Individual gene markers (total: 10)
2. Simple ratio (1 gene / 1 gene, total: 12 ratios)
3. All possible combinations of numerator and denominator (total: 44 ratios)

ANALYTICAL CHALLENGES

Use of gene ratios to stratify survival outcome has been successful in some cancer studies but limitations

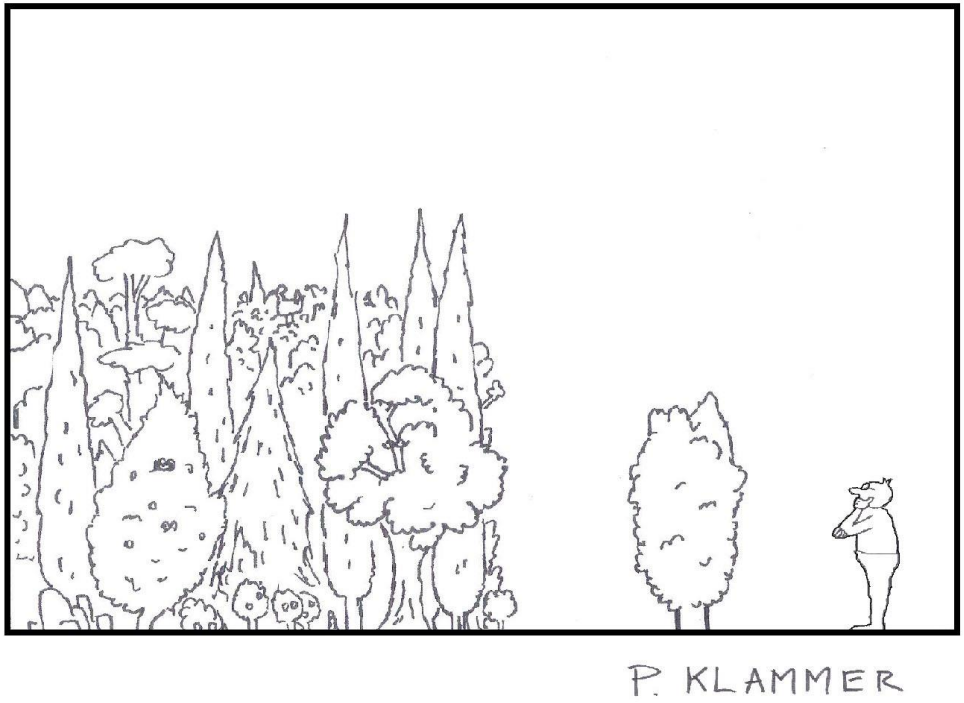
- **Define optimal cut-off ratio to segregate good and poor outcomes:** median is not satisfying as we expect ~30% mortality
- Assess all possible ratio combinations in an efficient way

Our aims

- Identify the most robust gene ratio among our gene candidates
- Determine optimal cut-off with appropriate statistical method
- Validate results in external cohort

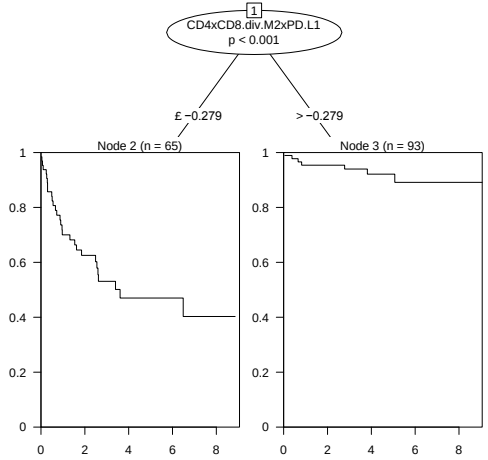
C Keane, K-A. Lê Cao, et al. and MK Gandhi (2015). Ratios of T-cell immune-effectors with tumour associated macrophages and PD-1/PD-L1 axis immune-checkpoint molecules, add to the predictive power of conventional prognosticators in diffuse large B cell lymphoma. *Lancet Haematology (08/2015)*

SEE THE FOREST FOR THE TREES

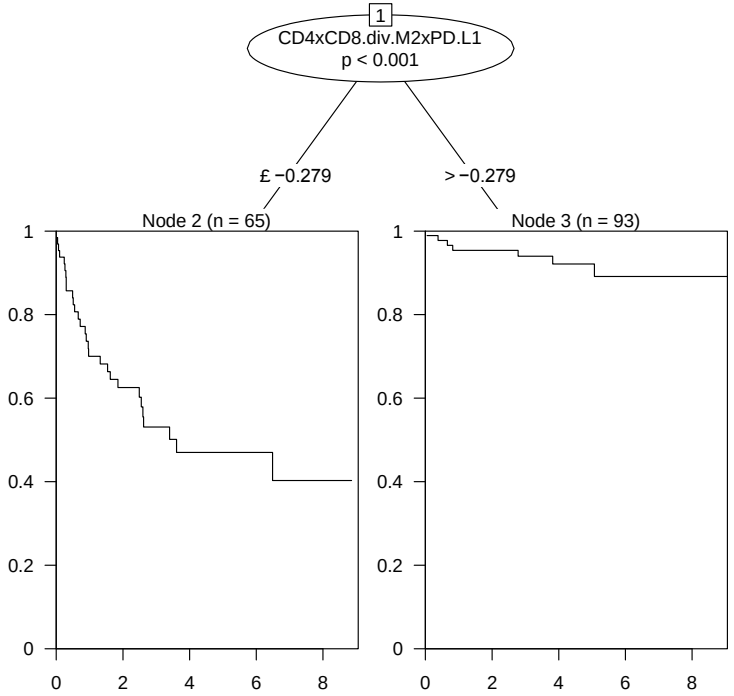


Tree-based survival models

- **Input**: gene expression ratio and survival outcome
- **Output**: stratify high vs. low risk patients with optimal ratio cut-off (‘best split’) based on entropy criterion



BEST SPLIT IN SURVIVAL TREE



Hothorn et al (2006), *Unbiased recursive partitioning: A conditional inference framework.* J of Comp Graph Stat. **15**(3)
R package `party`

Segregation rule based on patient partitioning for each ratio

- If ratio value **≤** best split, then patient classified as ‘high risk’ (LHS of tree)
- If ratio value **>** best split, then patient classified as ‘low risk’ (RHS of tree)

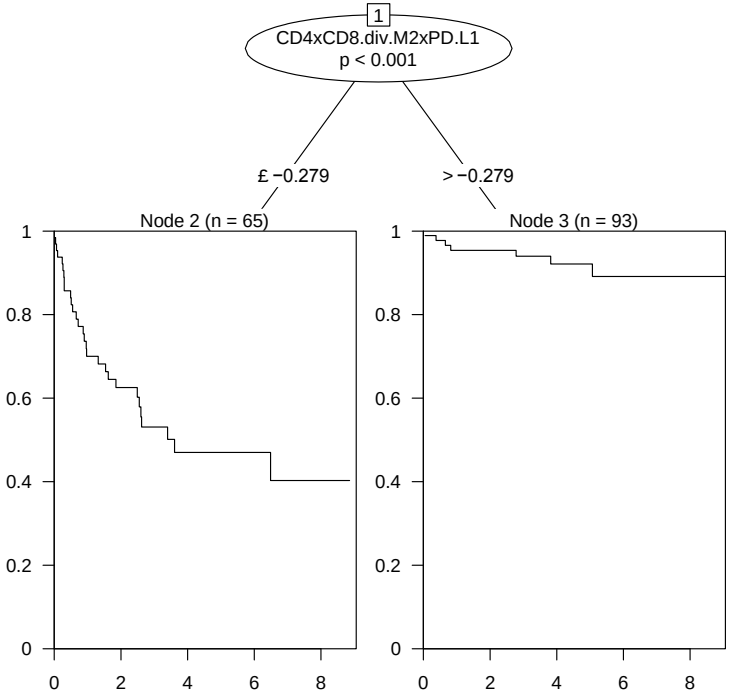
Discovery cohort (Nanostring)

- log rank test to assess differences btw the two survival curves obtained from tree + correction for multiple testing.

Test cohort (Affymetrix)

- Apply same split as in discovery data set, log rank test + correction for multiple testing.

ASSESSING VARIOUS COMBINATIONS



high-risk
n = 65

low-risk
n = 93

Single markers

- e.g. CD163, CD68, $p = 1$

Simple ratio

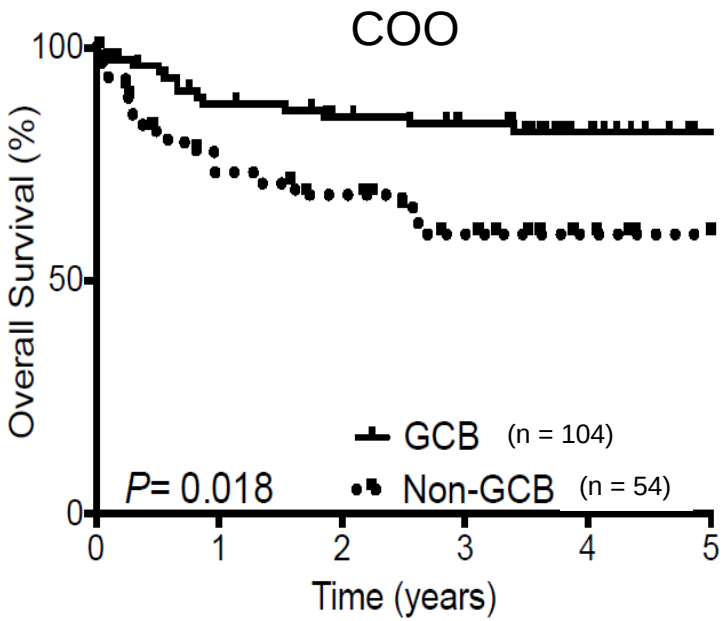
- e.g. M2 = CD163/CD68, $p = 0.0056$

Best combination identified with tree-based survival model

- (CD4 x CD8)/(M2 x PD-L1), p <0.0001
- best split = -0.279

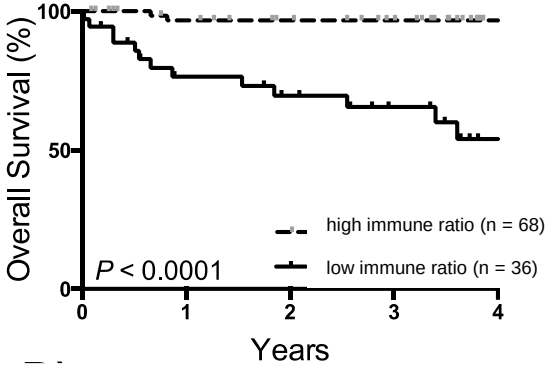
OUR IMMUNE RATIO ADDS TO CONVENTIONAL PROGNOSTICATORS

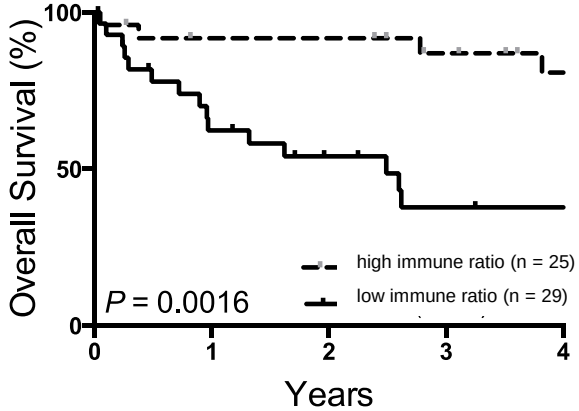
Cell of origin (COO) classifies
- germinal centre B-cell (GCB)
- non-GCB



GCB + immune ratio

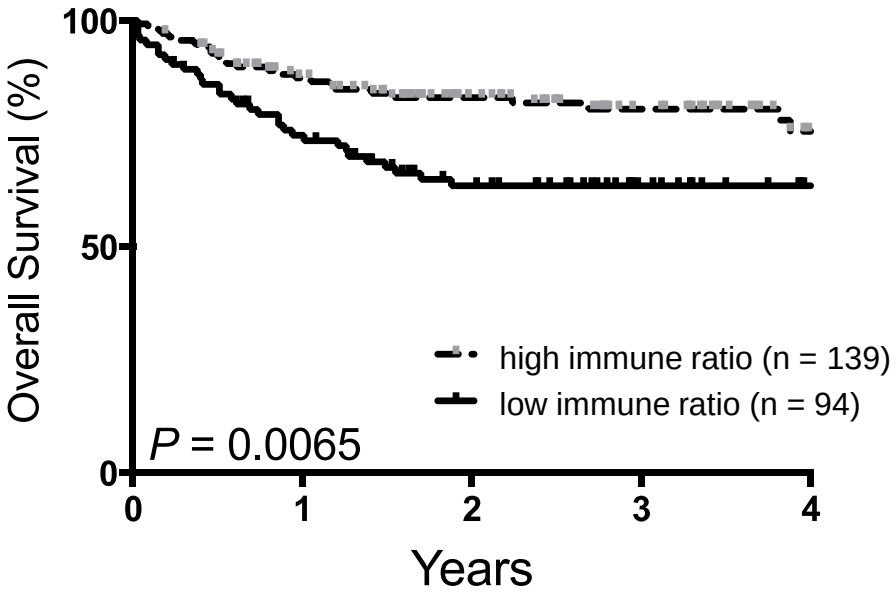
nGCB + immune ratio





40% of patients in high risk identified

VALIDATION IN EXTERNAL COHORT



Affymetrix gene expression study

1. Calculate our immune ratio (CD4 x CD8)/(M2 x PD-L1)
2. Set up our best split -0.279
3. Apply our segregation rule

- ~60% of patients assigned to high risk
- Multivariate Cox regression confirms immune ratio is independent from prognosticators COO and IPI

WHAT IF WE HAD USED A MEDIAN CUT-OFF?

# ratios with $p \leq 0.05$ (log rank test)	Median cut-off	Survival tree cut-off
Discovery Nanostring	21	54
Validation Affymetrix	0	4

Further work / Discussion

- fresh vs. frozen samples have an influence on validation
- improve results with a confidence interval for best split
- not shown: experimental validation successful in on a 3rd cohort (n = 160, flow cytometry and i-PET/CT outcome)

CONCLUSIONS

Our immune ratio

- Based on biological hypothesis
- Complements conventional prognosticators
- Identifies 40% of patients with poor outcome
- Validation in two external cohorts
- May help identify better target drugs (e.g. PDL-1) for patients not responding to R-CHOP. Clinical trial? (patent submitted).

Ratios for big data? and my (statistician) point of view

- Address the issue of platform variability
- Combination of genes vs. single gene biomarker approach is beneficial
- Could be expanded to other cancer studies genomewide

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