Molecular analysis of primary melanoma T cells identifies patients at risk for metastatic recurrence

Pruessmann *et al*. 2020 Nat Cancer. doi: 10.1038/s43018-019-0019-5

Presented by Kim Dill-McFarland

2021.06.07 @kdillmcfarland

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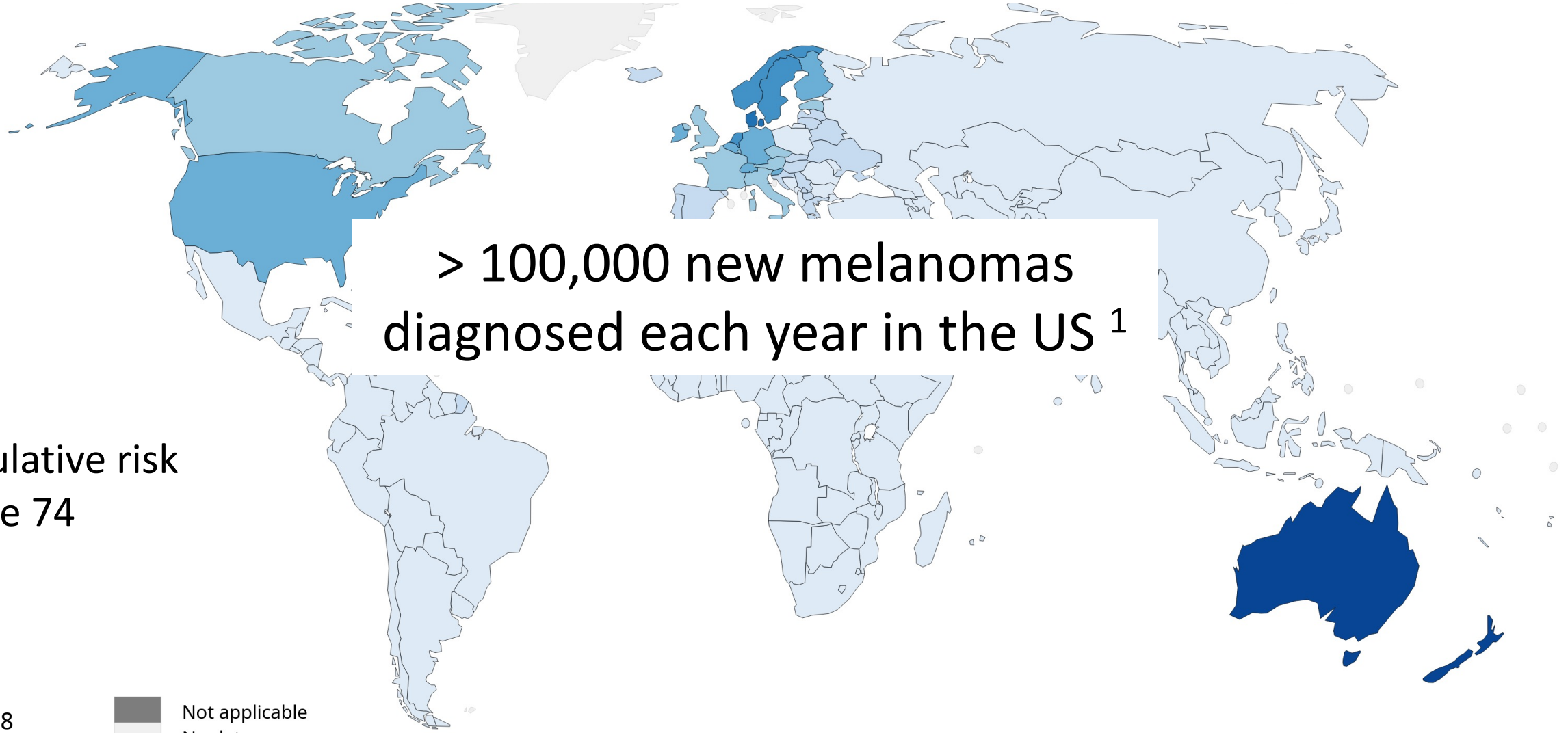
2020 Melanoma cumulative risk of incidence



Data source: GLOBOCAN 2020
Graph production: IARC (http://gco.iarc.fr/today)
World Health Organization

Melanoma risk factors [1]

- Ultraviolet (UV) light exposure
  - Especially sunburn
- Age
- Skin irregularities like freckles and moles
  - < 30% of skin cancers originate in a mole
- Skin color
  - Rates of melanoma are lower in non-Hispanic Black compared to White populations (1:25)
  - Mortality due to melanoma is disproportionately high in non-Hispanic Black populations (1:10) [2]

Recurrence

- Surgical removal often effective but…
- Metastatic recurrence common at > 20% of primary melanomas within 5 years [3]

- Prediction of recurrence can aid monitoring and treatment plans

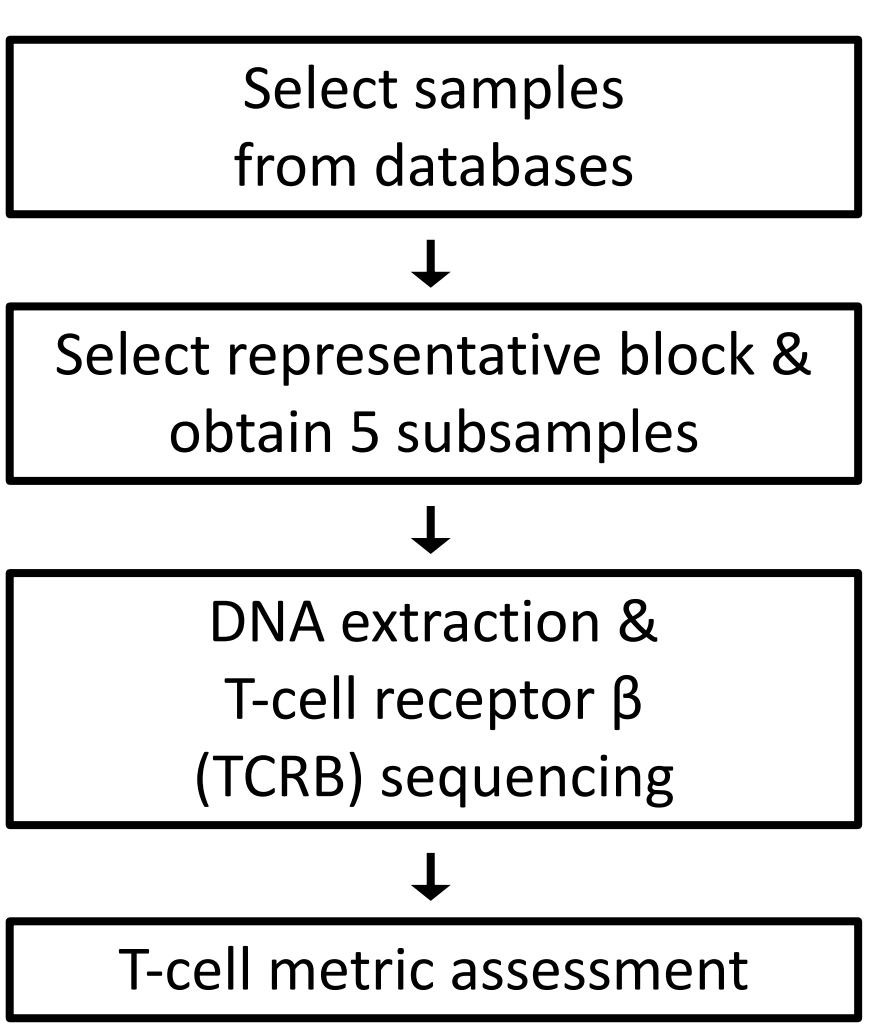
T cells as predictors of recurrence

- Melanomas cause an adaptive immune response [5]
- Tumor-infiltrating lymphocytes associated with less recurrence [6]
- T-cell receptor clonality predicts response to some therapies [7]

Primary hypothesis

T-cell fraction (TCFr) and / or T-cell clonality improves prediction of primary melanoma recurrence compared to current clinical measures alone

Design



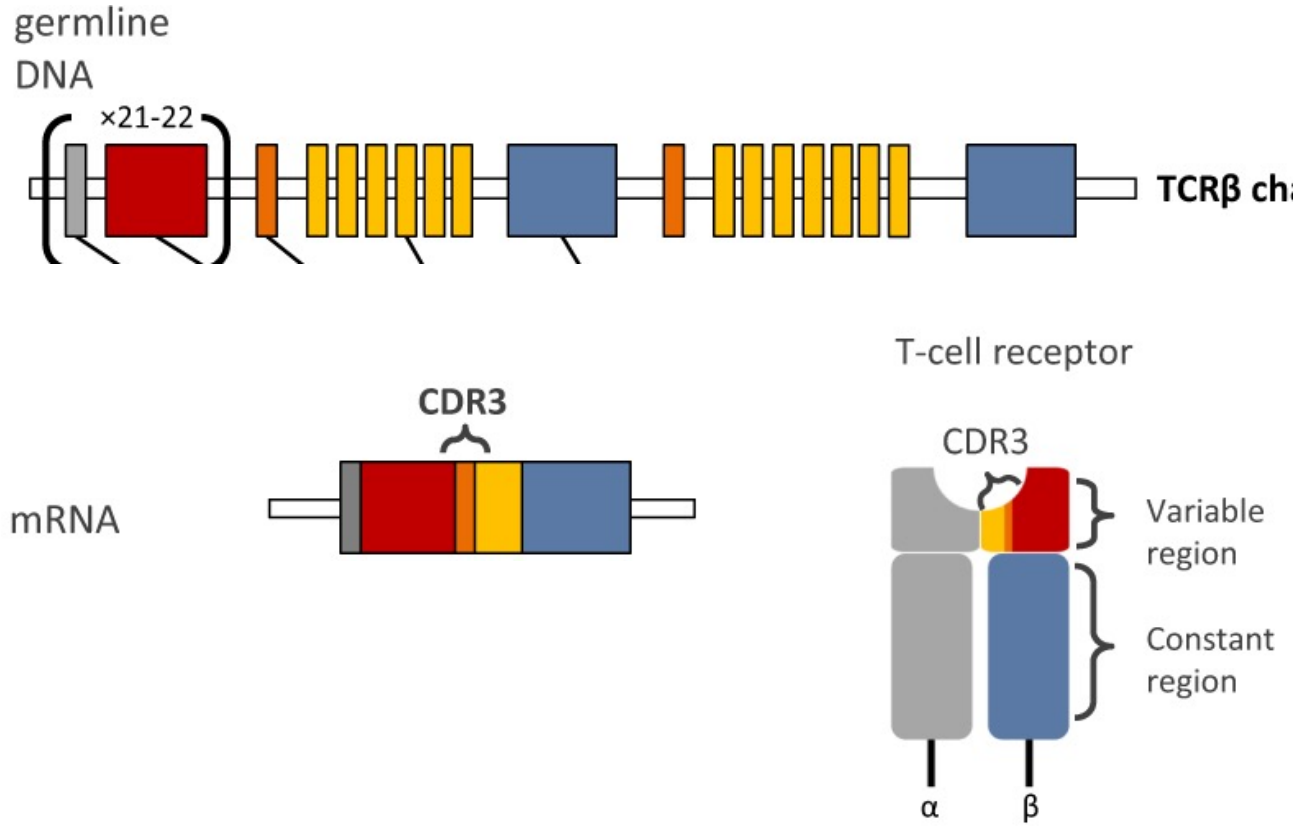
- Primary melanoma stage I – III
- Complete excision biopsies available
- 50% survival within varied tumor thickness groups

- Verify tumor presence before & absence after excision

n = 337

TCFr n = 199
Clonality n = 158

TCRB sequencing targets CDR3



modified from 8

T-cell metrics

- T-cell clonality
  - > 100 T cells per sample
  - Simpson measure $= \sqrt{\sum p_i^2}$ p_i = abundance of clone i / total clones
  - 0 = highly polyclonal, high-diversity

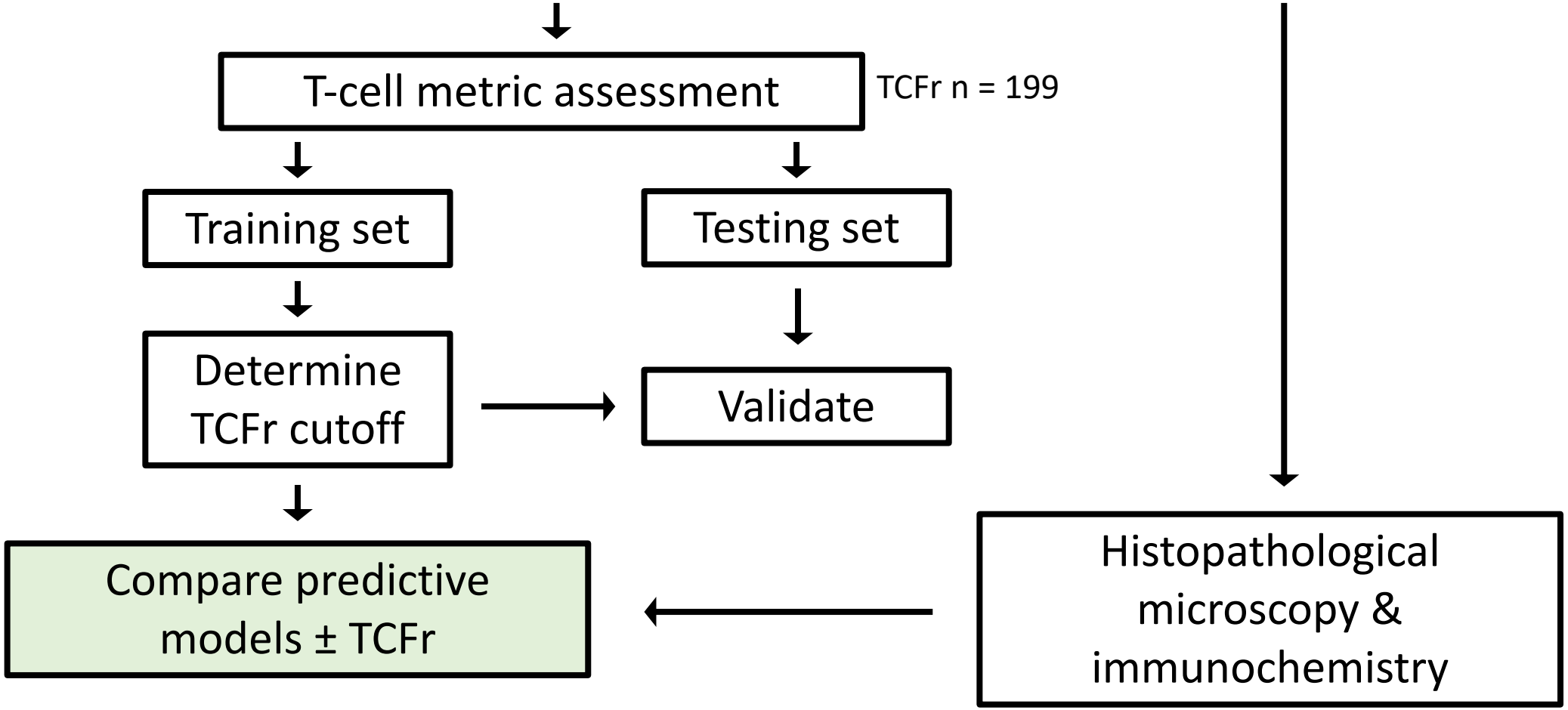
- T-cell fraction (TCFr)
  - > 500 nucleated cells per sample
  - Total T cells / total nucleated cells

T-cell fraction (TCFr) as a predictor of recurrence

TCFr associated with recurrence independently from other variables

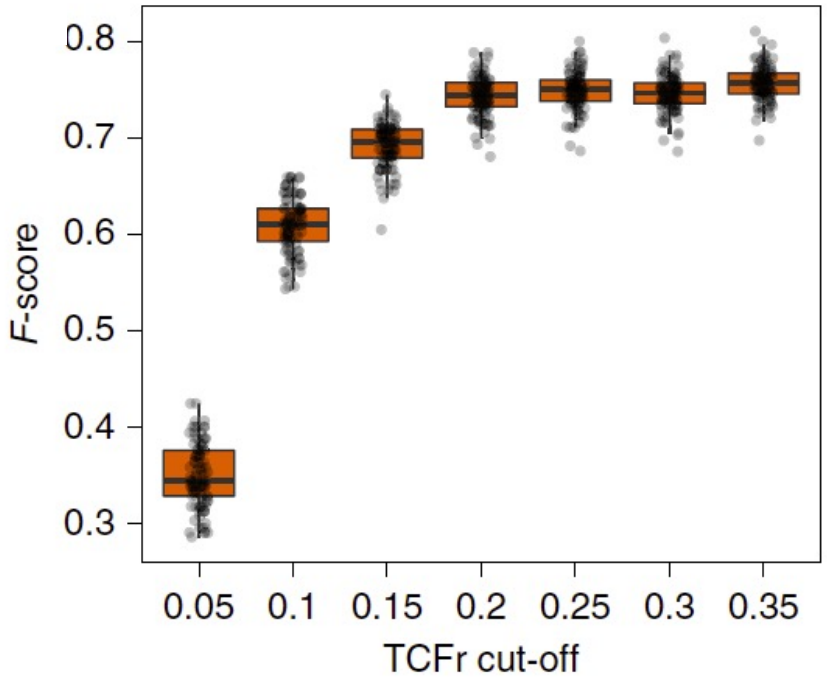
	TCFr association P-value
Recurrence	< 1E4
Survival	< 1E4
Tumor thickness	0.52
Age	0.76
Sex	0.41
Nodal disease	0.15
Ulceration	0.89
Mitotic rate	0.41

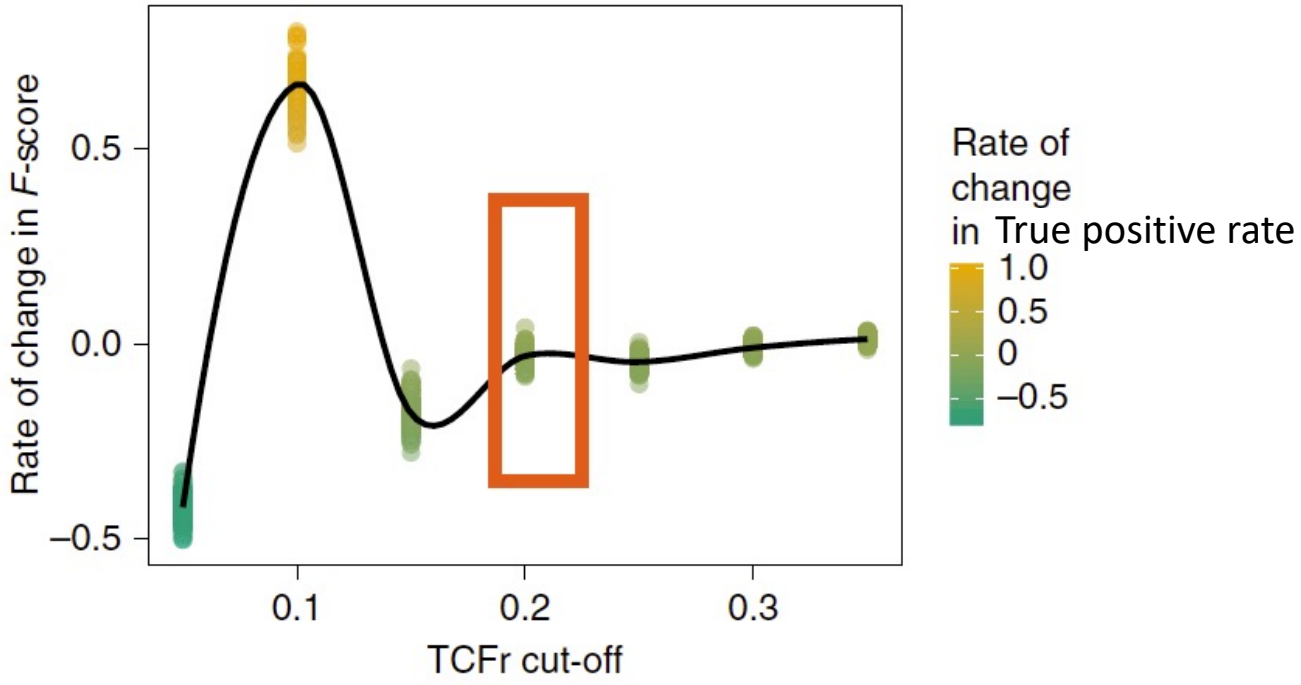
Design


T-cell metric assessment
TCFr n = 199
Training set
Testing set
Determine TCFr cutoff
Validate
Compare predictive models ± TCFr
Histopathological microscopy & immunochemistry

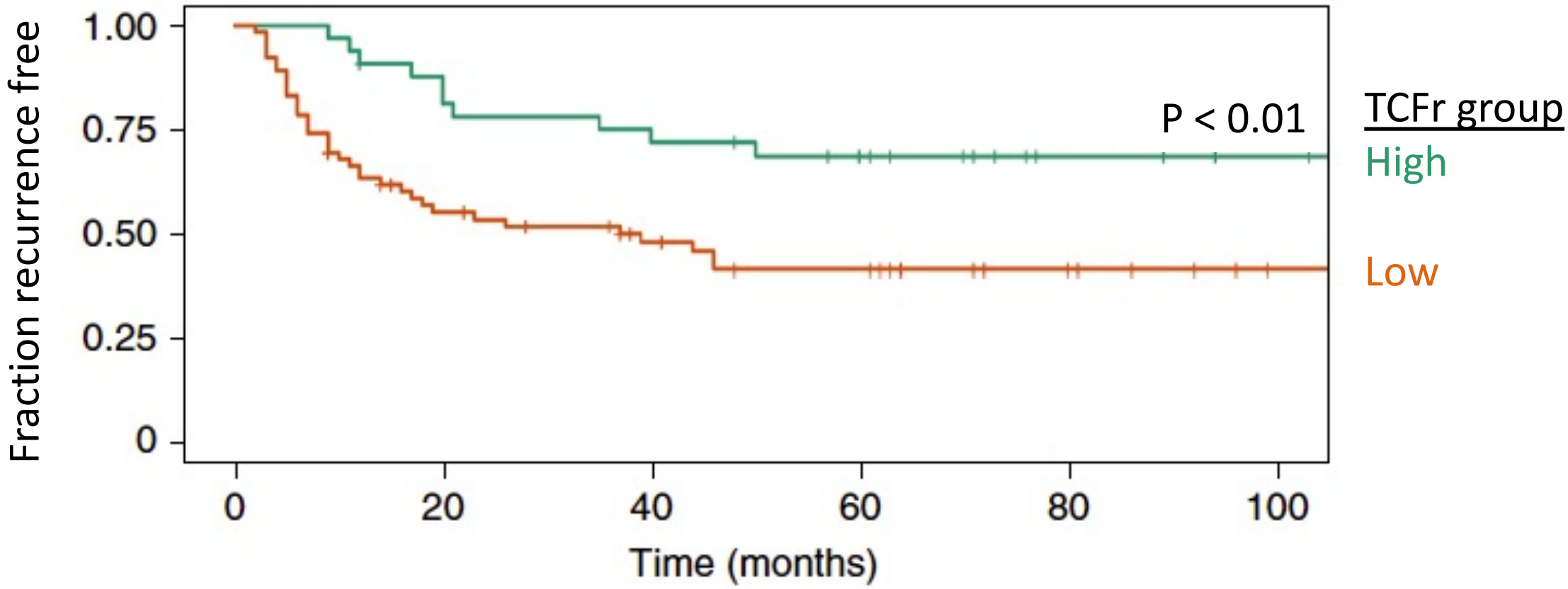
TCFr cutoff to best predict recurrence

- 80% subsample bootstrapping
- 0.05 incremental TCFr cutoffs
- Minimize change in true positive rate (TPR) and F-score $= 2\times\frac{precision\times recall}{precision+recall}$

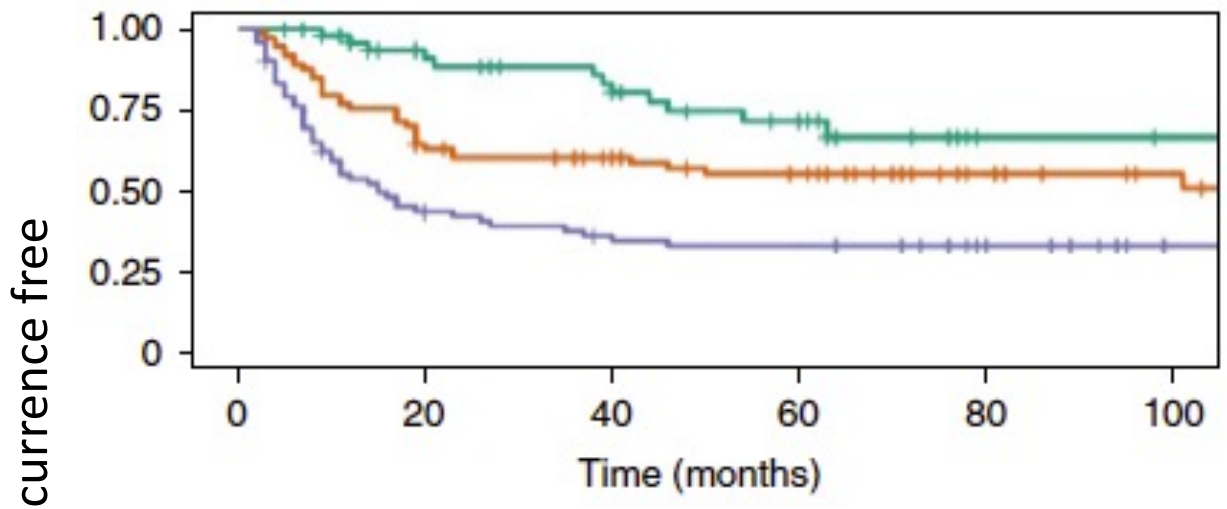




20% TCFr validated in testing set



20% TCFr improves recurrence prediction

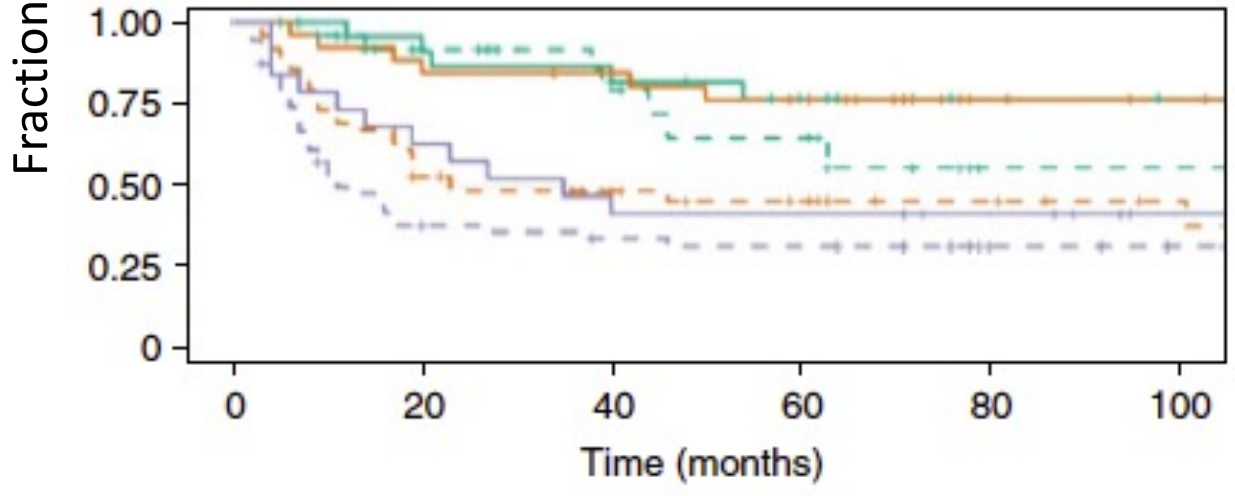


Tumor thickness

1-2 mm

2-4 mm

> 4 mm



Tumor thickness + TCFr

— High TCFr

1-2 mm 2-4 mm

> 4 mm

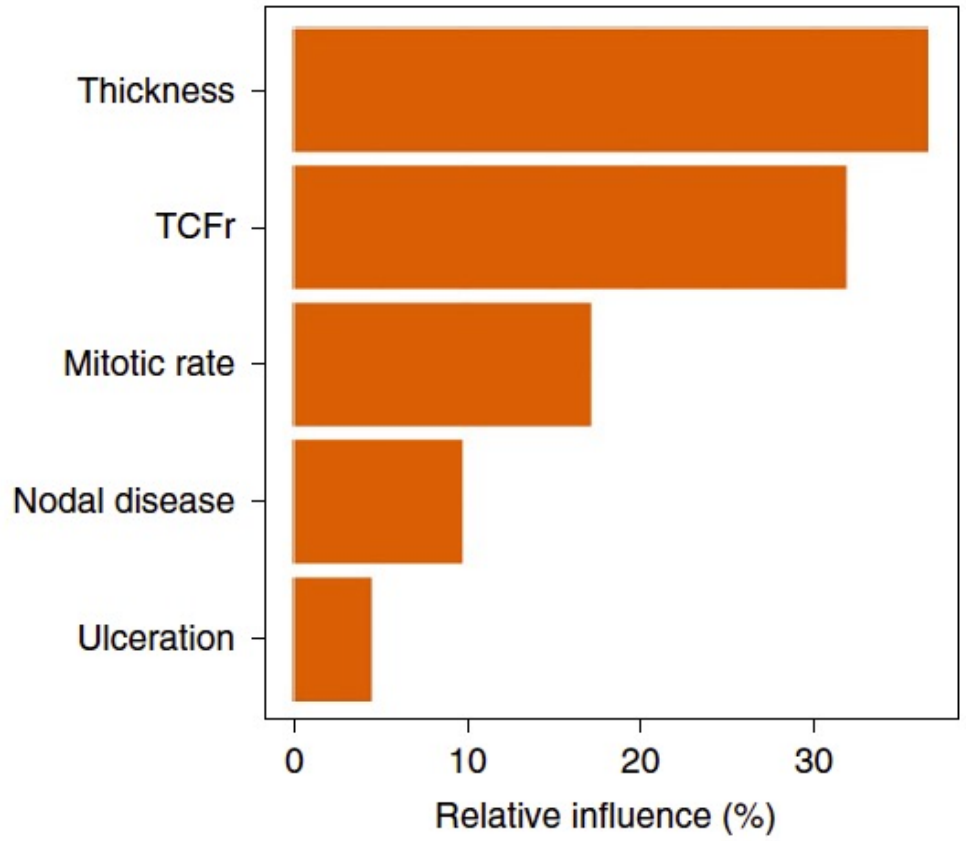
- - Low TCFr

1-2 mm

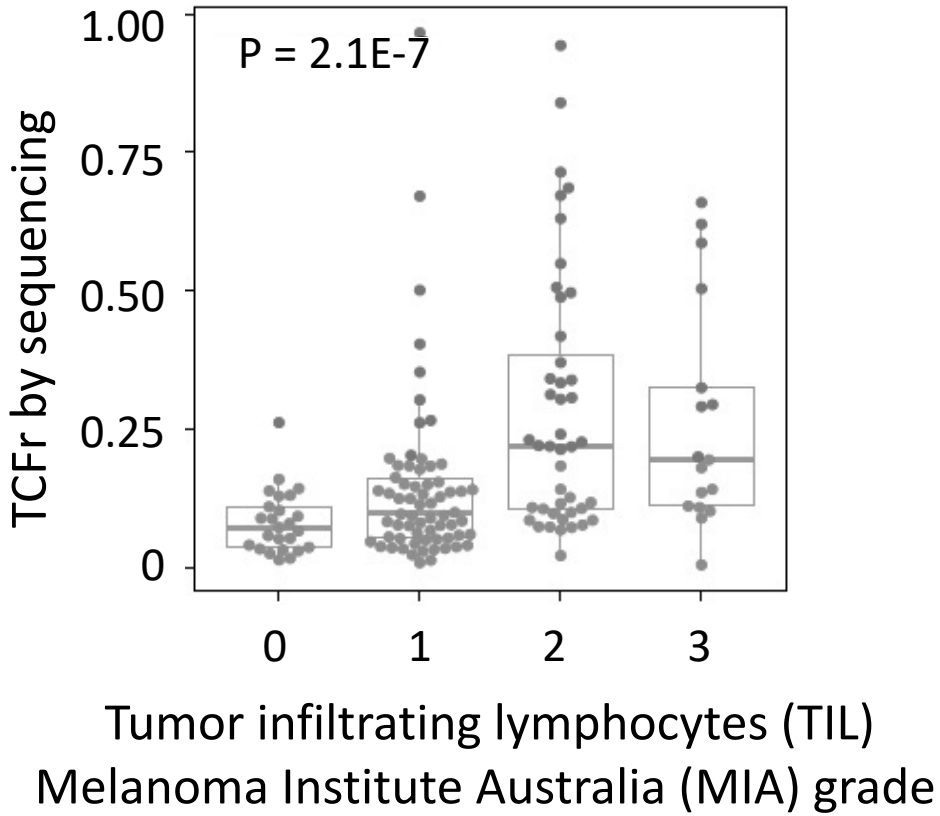
2-4 mm

> 4 mm

20% TCFr among top predictors of recurrence



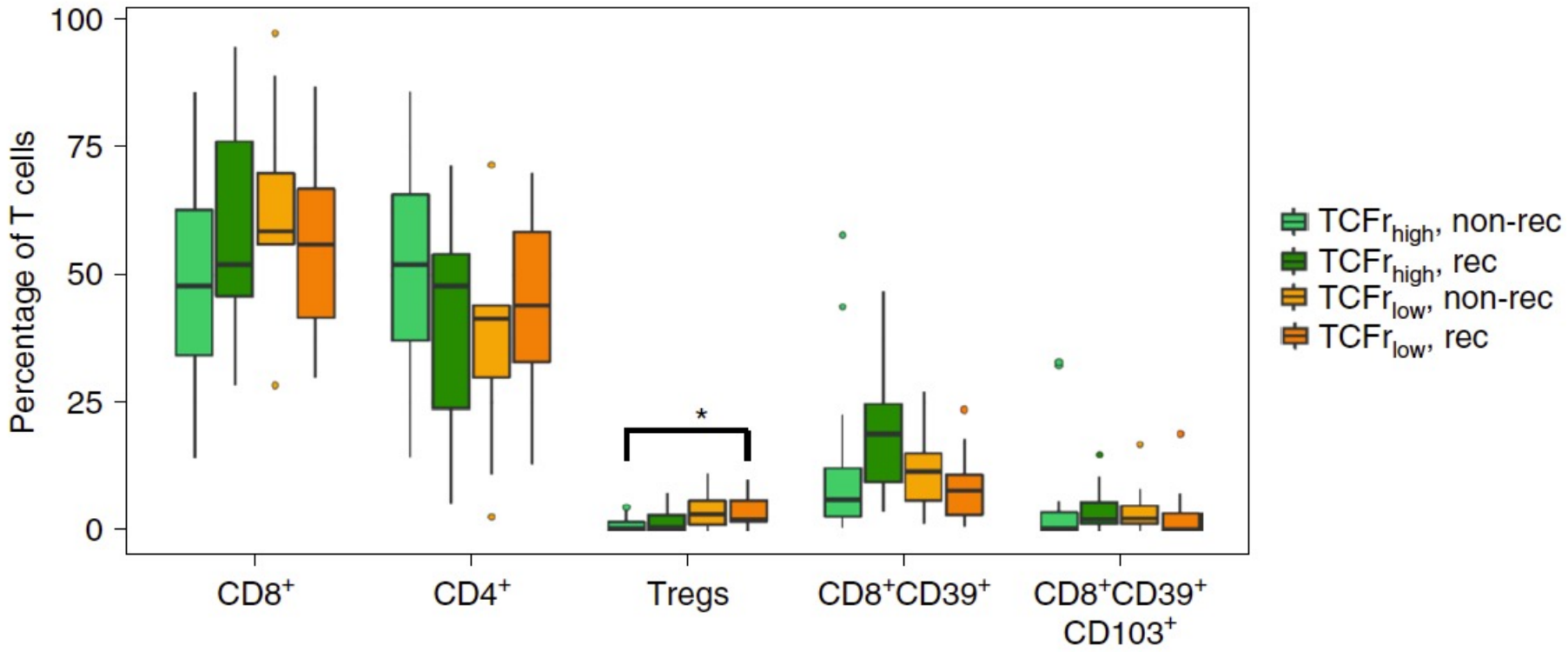
TCFr correlated with more traditional measures but TCFr is more predictive



Analysis of deviance $P < 0.01$
TCFr model vs

- TIL MIA grade
- TIL briskness

TCFr correlated with some T-cell subsets but TCFr is more predictive



None predict recurrence, P > 0.05

Conclusions

- T-cell fraction (TCFr) is a unique measure not correlated with a number of current clinical measures
- TCFr less than 20% is associated with higher primary melanoma recurrence
- 20% TCFr improves recurrence prediction in conjunction with current clinical measures, particularly tumor thickness
- Treg proportion within TCFr may further improve prediction

Primary hypothesis

T-cell fraction (TCFr) ~~and / or T-cell clonality~~ improves prediction of primary melanoma recurrence compared to current clinical measures alone

Next steps

Incorporate TCFr measures with

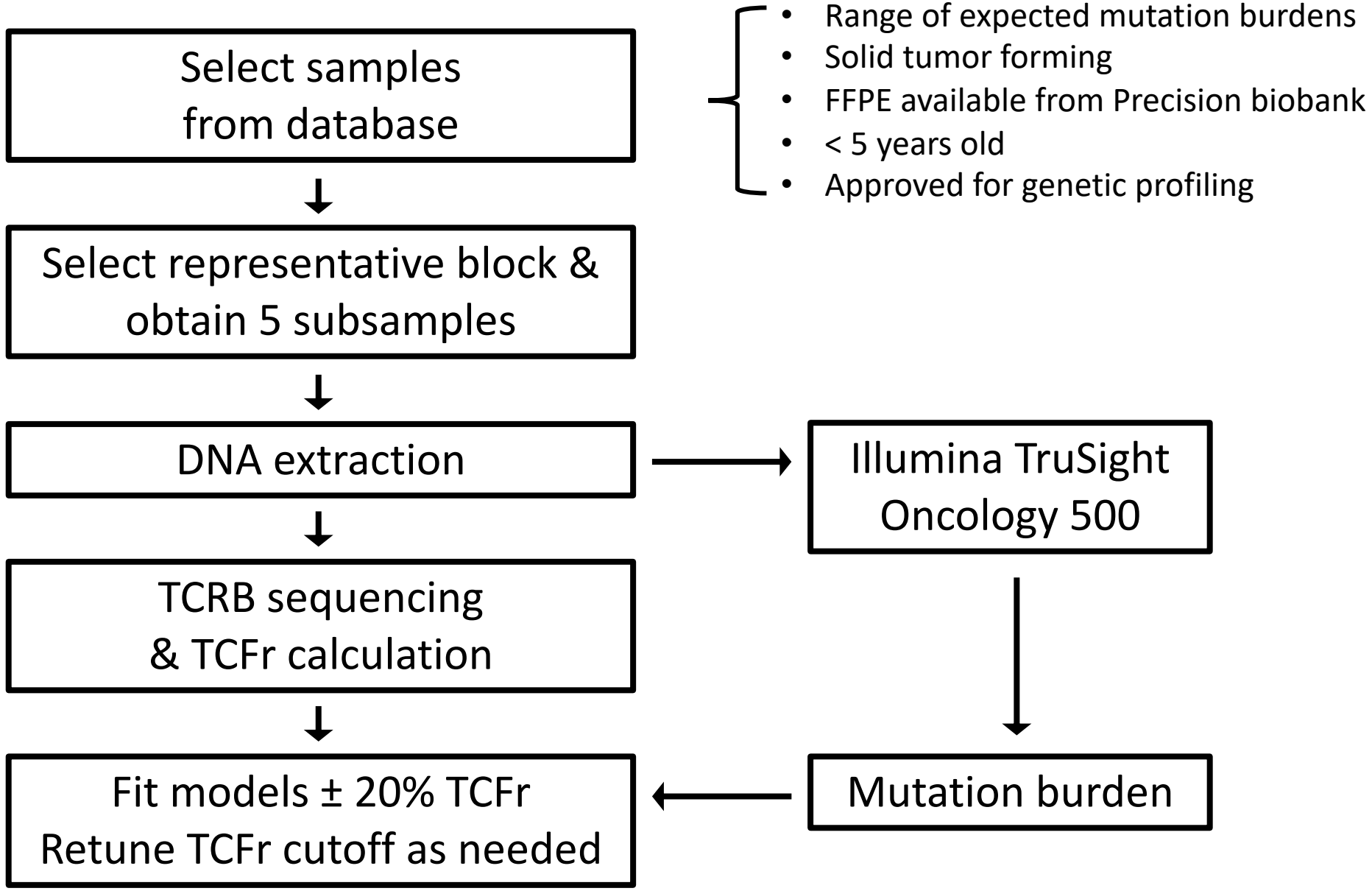
- anti-PD-1 blockade (or other treatment) trial
- metastatic melanoma
- < 1 mm melanoma

My next step proposed

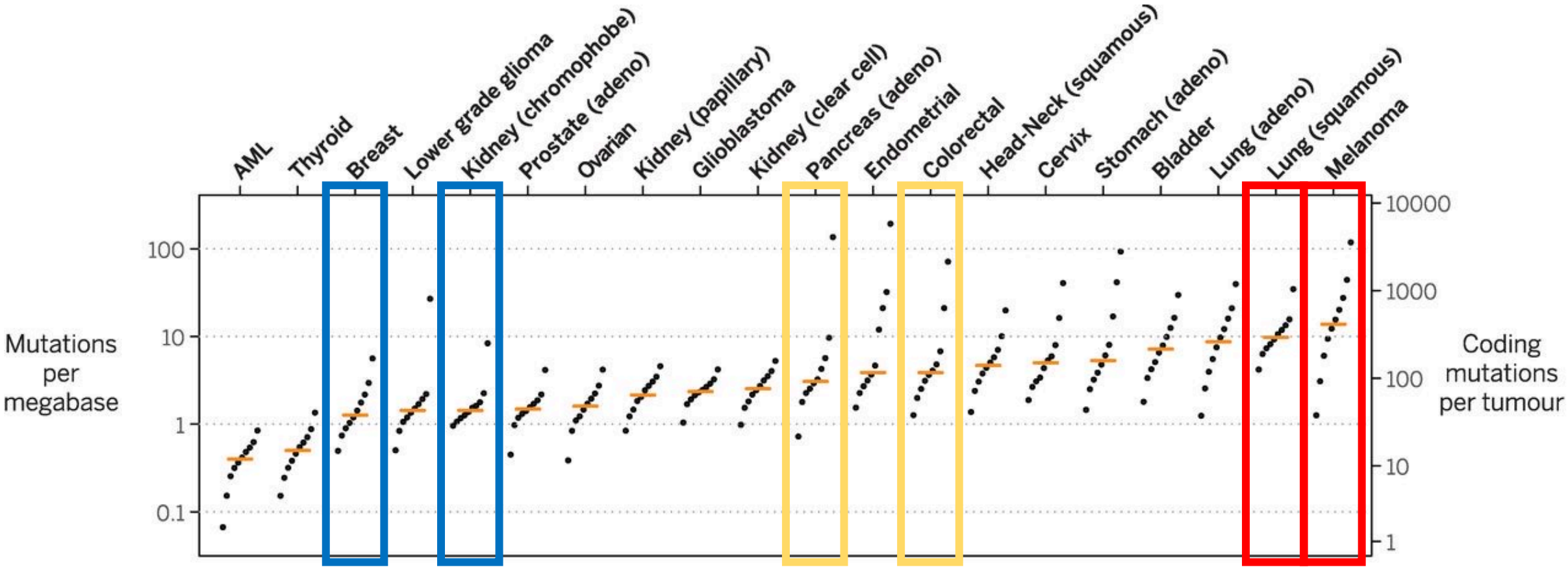
Hypothesis

T-cell fraction (TCFr) improves prediction of high-mutation but not low-mutation burden cancer outcomes

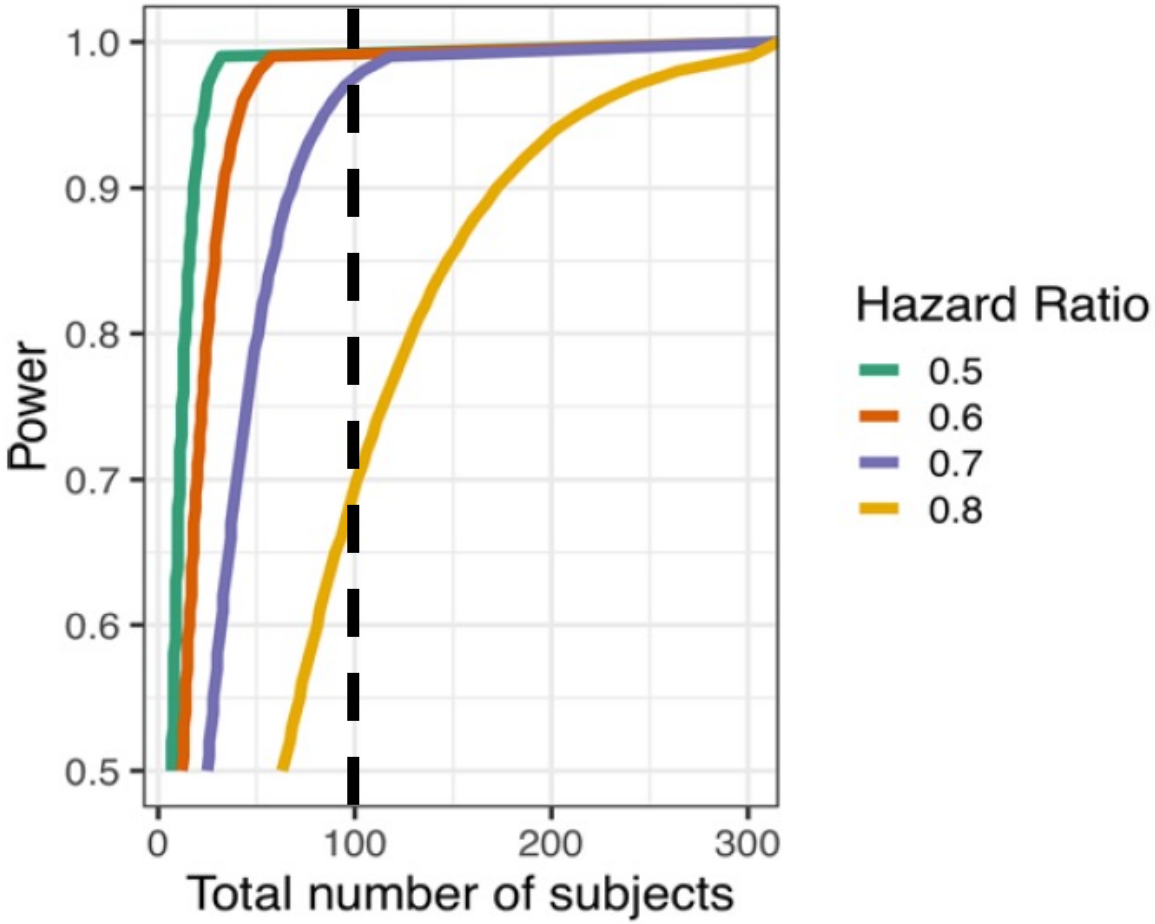
Design


Select samples from database
Range of expected mutation burdens
Solid tumor forming
FFPE available from Precision biobank
< 5 years old
Approved for genetic profiling
Select representative block & obtain 5 subsamples
DNA extraction
Illumina TruSight Oncology 500
TCRB sequencing & TCFr calculation
Mutation burden
Fit models ± 20% TCFr
Retune TCFr cutoff as needed

Cancer type selection



Sample size



- Expect high-burden hazard ratio near melanoma (0.8)
- Expect sample failure at mean 1 to 5-year-old samples (15%)
- Goal: 118 samples per cancer (after failure ≈ 100)

Potential variables

Outcome

- Recurrence
- Survival

Co-variate

- Common risks (thickness, location, etc)
- Demographics (age, sex, etc)
- Disease history

Explanatory

- TCFr (20%, continuous)
- Mutation burden (high/low, continuous)

Analyses

- Cox’s proportional-hazard model
  - Separate cancers vs combined analysis

  ± TCFr

  ± mutation burden

- Sparse partial least-squares regression (sPLS) [10]

References

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ARTICLES

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