OXFORD Molecular Diagnostics Centre

Oxford University Hospitals NHS
NHS Foundation Trust

Discovery of low level TP53 variants in CLL diagnostic samples using a custom designed NGS panel and the impact on clinical outcome.

Ria Hipkiss

Oxford Molecular Diagnostic Centre

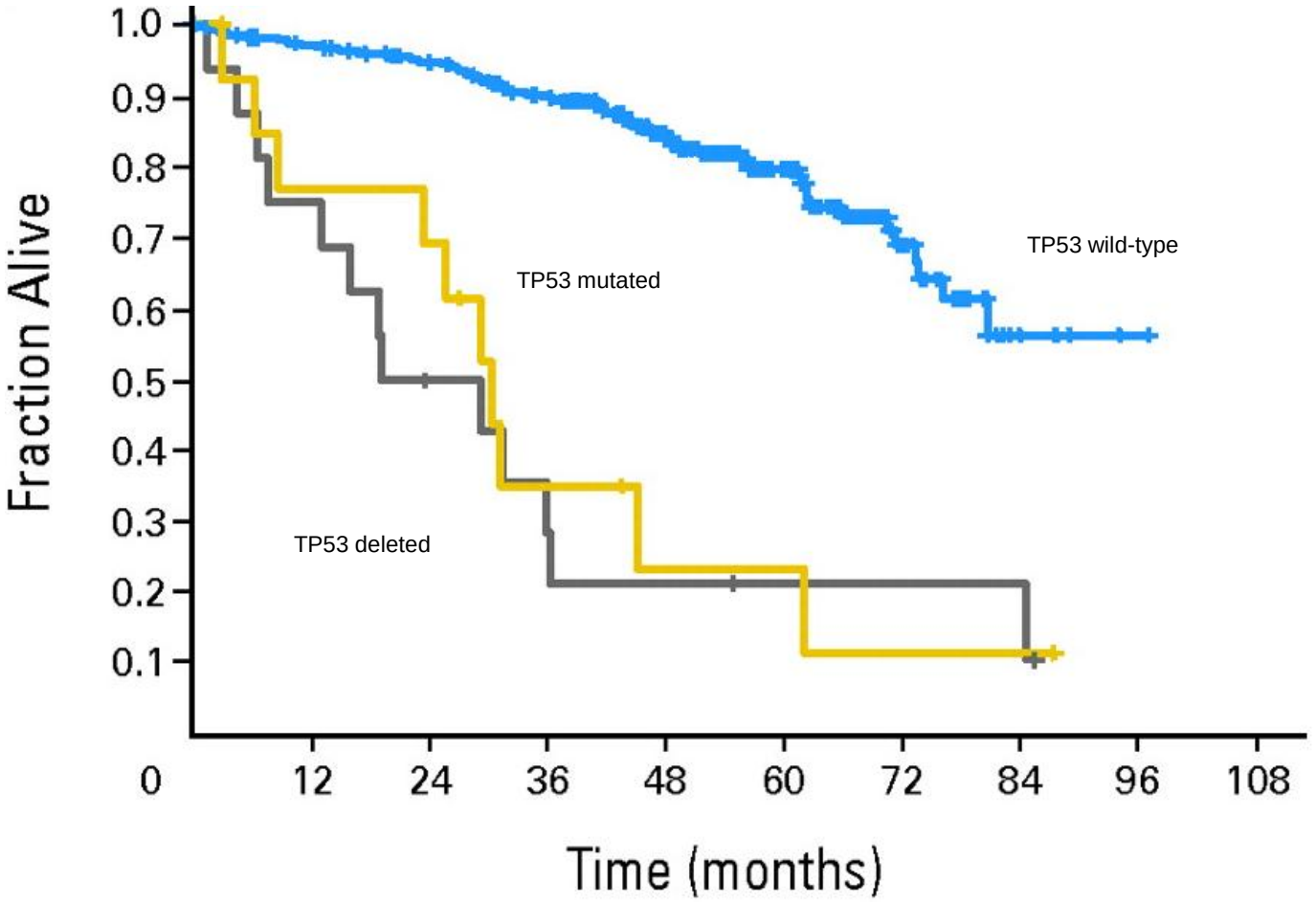
Oxford University Hospitals Foundation Trust

http://www.oxford-translational-molecular-diagnostics.org.uk/

Chronic Lymphocytic Leukaemia (CLL)

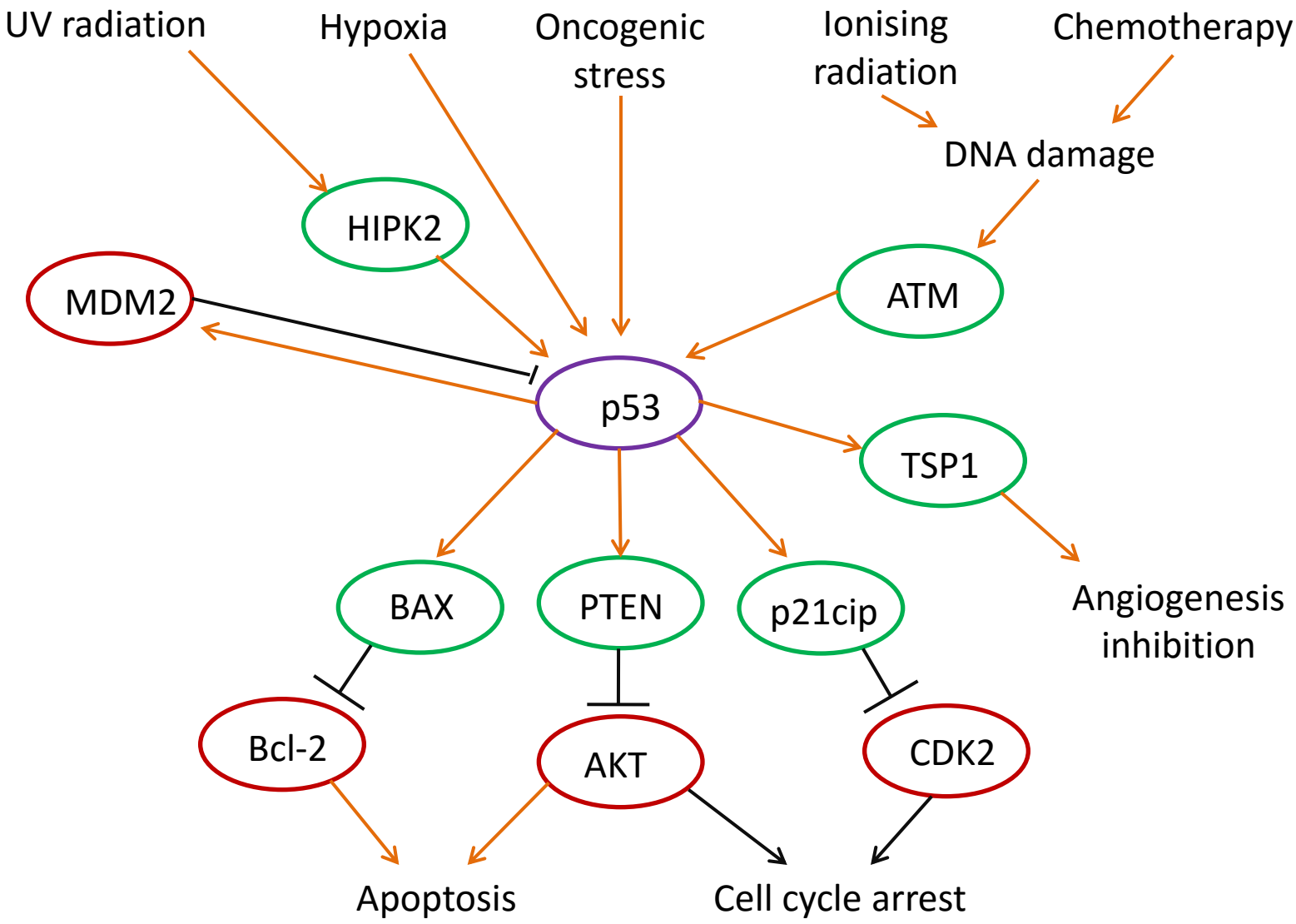
- Most common leukaemia in the Western world,
- Disease of elderly (median age 70),
- Median survival in excess of 10 years,
- Heterogeneous – wide variation in clinical outcome,
- Most important prognostic factor is detection of TP53 (17p13.1) gene aberrations.

Impact of TP53 Status on Survival



Zenz et. al. 2010. *Journal of Clinical Oncology*. 28(29), pp. 4473-4479

Biological Function of the p53 protein

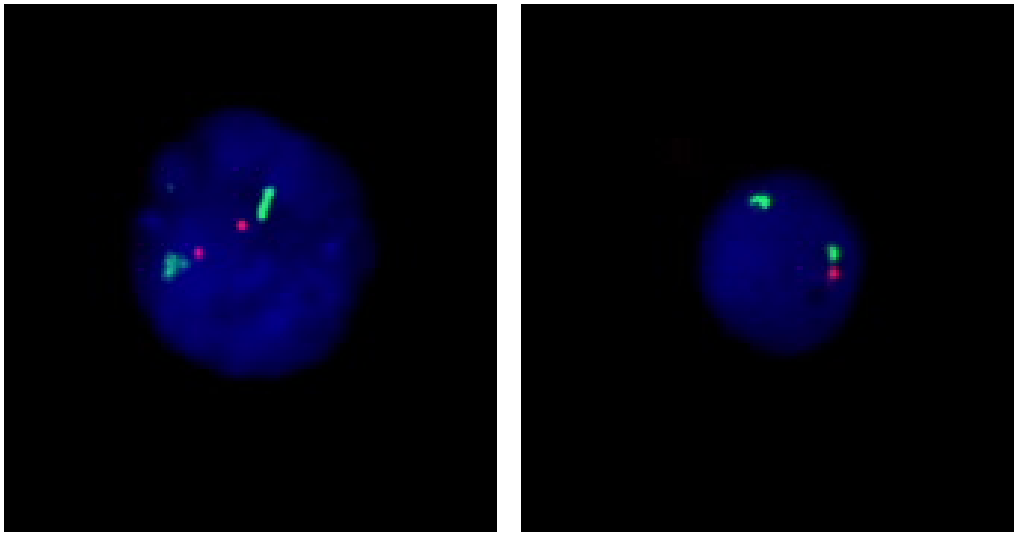


Treatment

- Conventional chemotherapy
  - Direct DNA damage
  - Effect mediated via TP53 pathway
  - Ineffective in patients with TP53 aberration
- Tyrosine Kinase Inhibitors (TKI) such as Ibrutinib
  - Mediate their effect via the B-Cell Receptor (BCR) pathway,
  - Highly effective in patients with TP53 aberration
- **Standard of care is to define TP53 status in all patients prior to initiating treatment**

Current Laboratory Practices

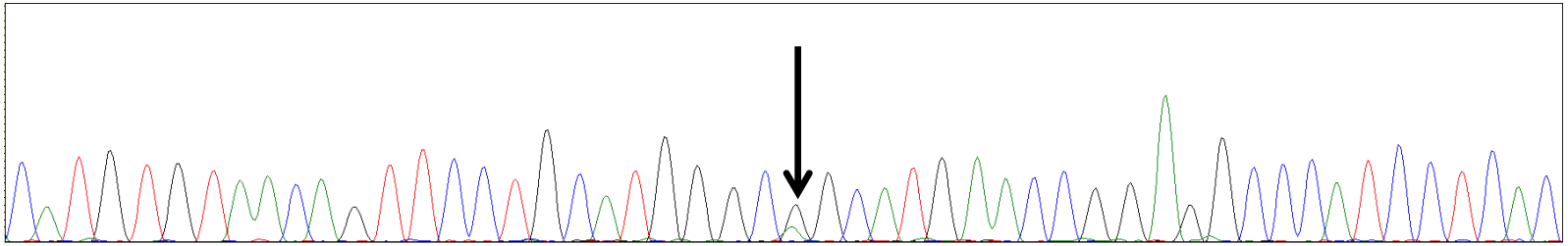
- Florescent in-situ hybridisation (FISH)
- Detection of whole gene deletion



Sellner, L. *et. al*. (2013). *Current Hematological Malignancies Rep*. 8, pp. 81–90.

Current Laboratory Practices

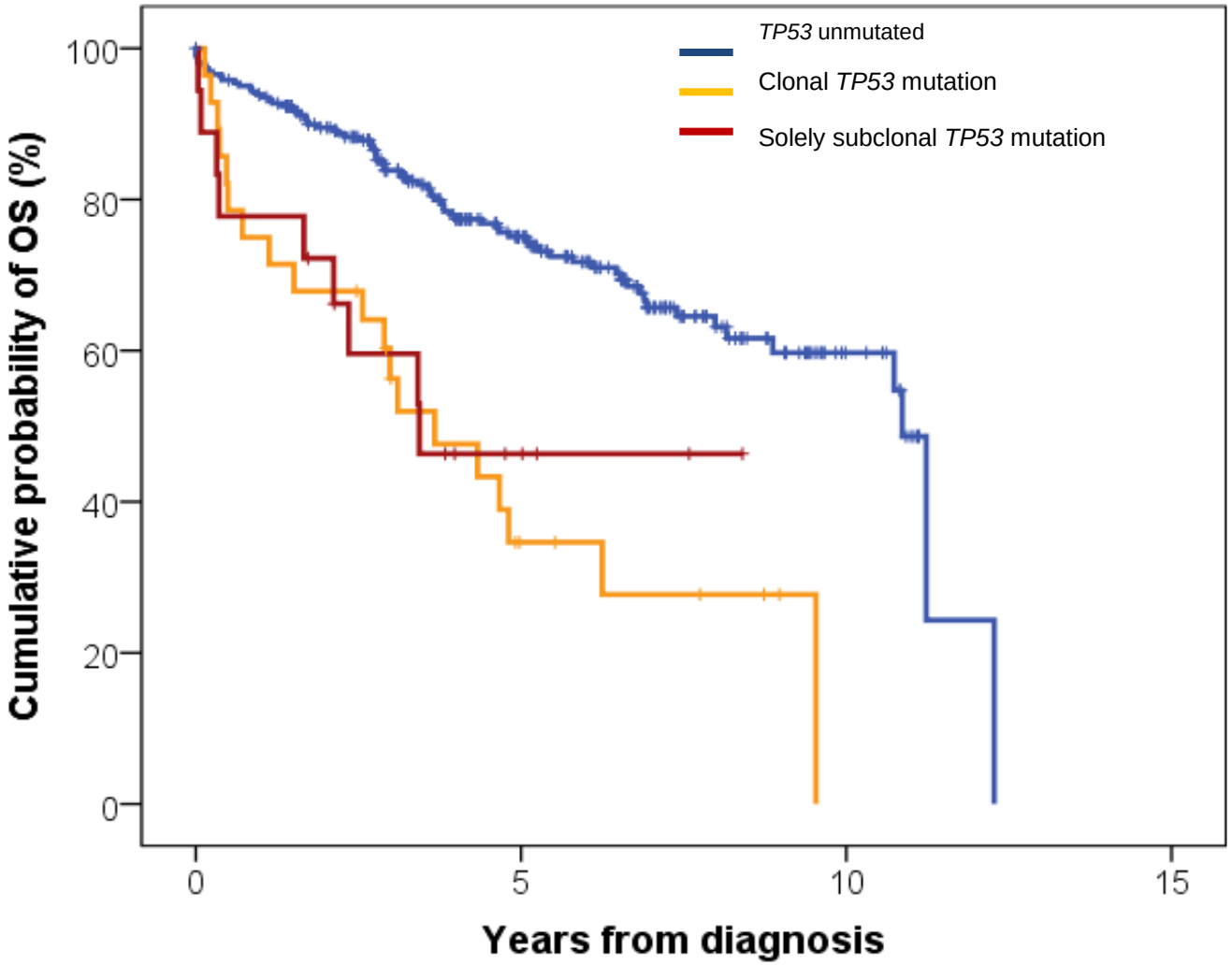
- Sanger sequencing of minimum exons 4-9 covering at least 90% of mutations reported,
- Identification of SNVs and InDels



- Can only reliably detect 20% variant frequency against the wild-type background.

Pospisilova, S. *et. al.* (2012). *Leukemia*. 26, pp. 1458-1461.

Impact of Low Level TP53 Mutations on Survival



Rossi et. al. 2014. *Blood*. 123(14), pp. 2139-2147

Targeted Sequencing Custom Amplicon panels by NGS

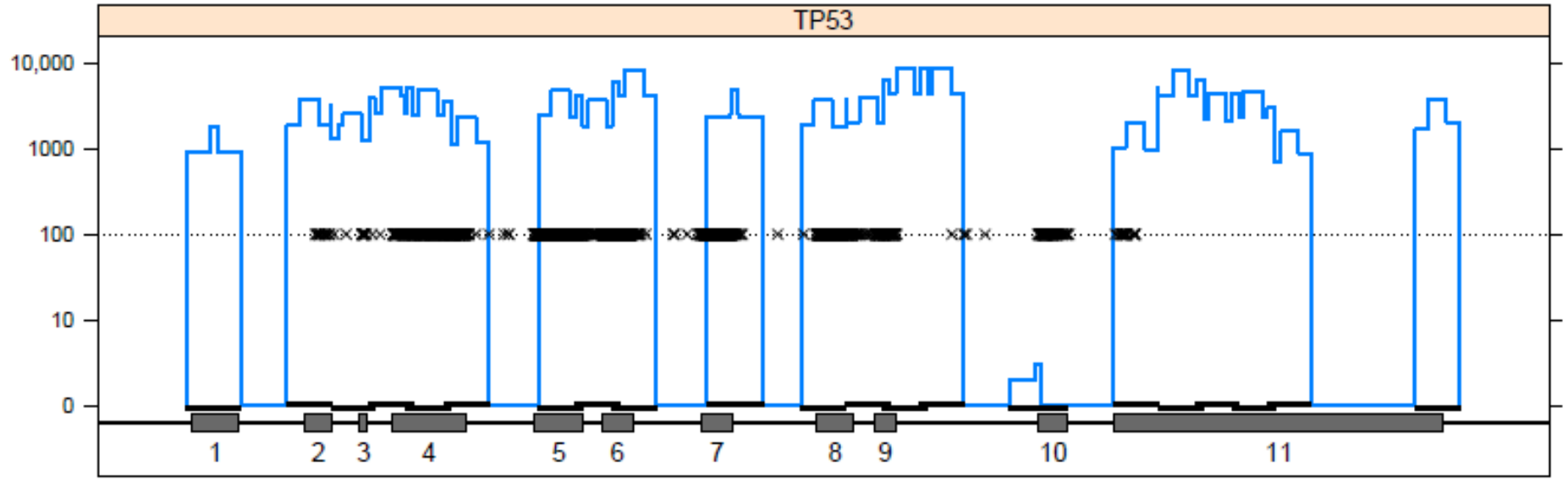
- **TruSeq Custom Amplicon (TSCA) CLL: TP53**
  - Theoretical coverage : 100% (Exons 1- 11)
  - Theoretical Sensitivity : 1%
- **Cohort:**
  - 90 routine clinical samples
    - 30 positive for TP53 by Sanger
    - 60 negative for TP53 by Sanger (also negative by FISH for deletions)

Targeted Sequencing Custom Amplicon panels by NGS

- Assay:
  - TSCA v1.5
  - MiSEQ (2x150)
- Bioinformatics:
  - Somatic Variant Caller (1% threshold)
  - Platypus
  - VariantStudio - Filter settings:
    - minimum 100 reads
    - minimum quality 100.

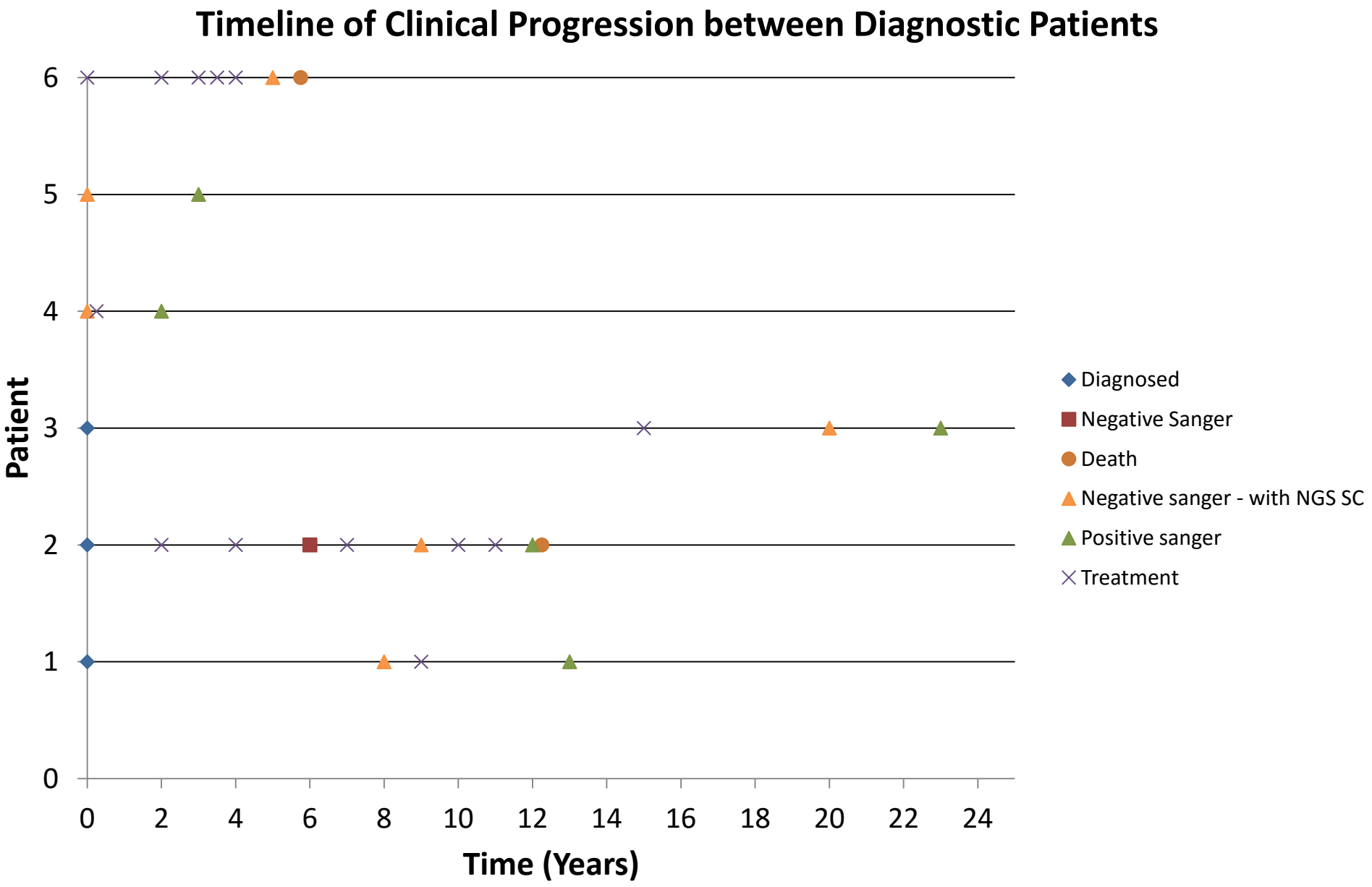
Panel Coverage

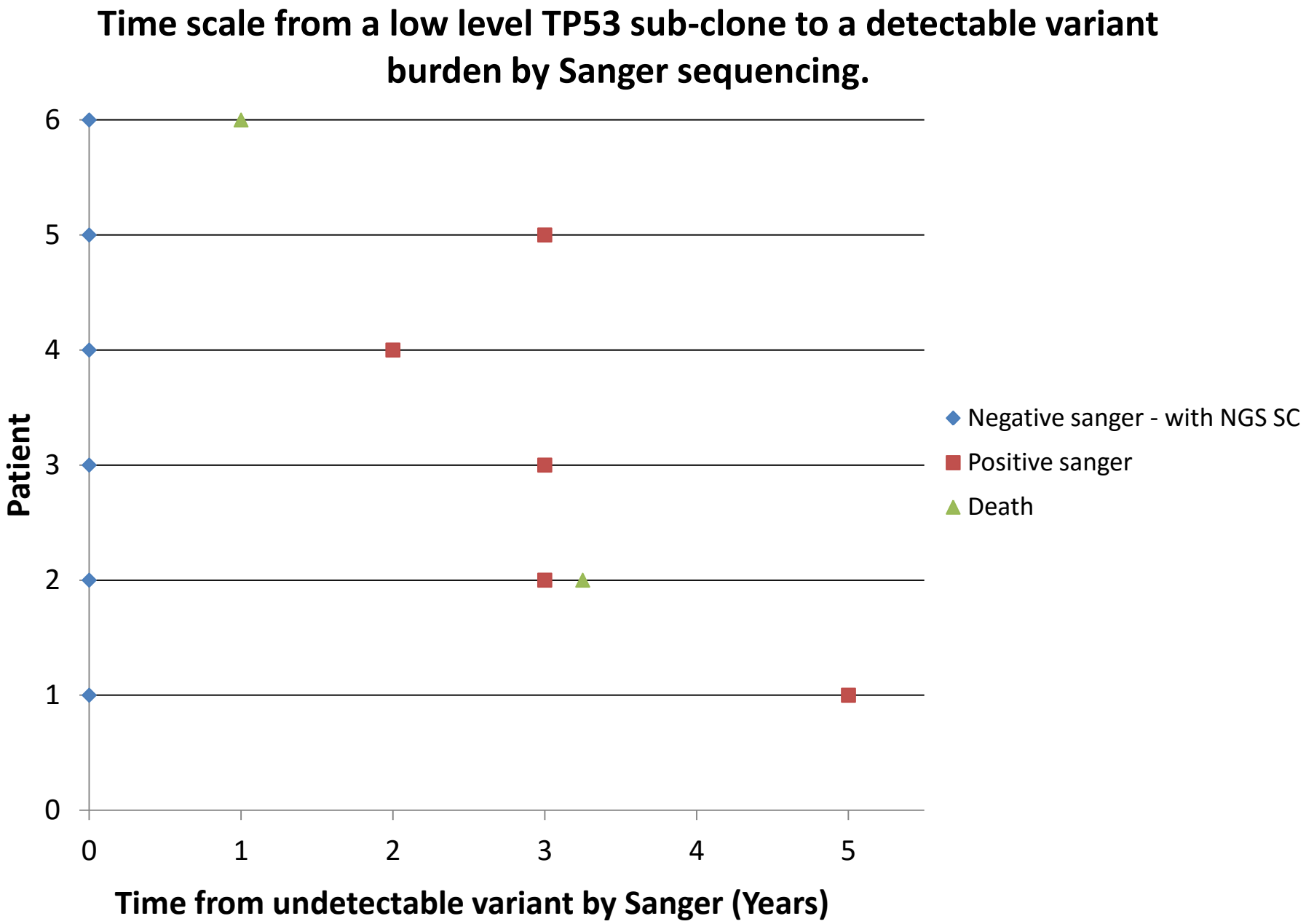
- Coverage: 93.6 ± 8.8 % (mean ± SD)
- Clinical Sensitivity: 96% of variants listed in COSMIC (v72)



Clinical Outcome of Low Level TP53 Mutation Burden

- Of the 60 negative TP53 samples (by Sanger) 6 were found to have a low level mutation by NGS
- Confirmed by alternative method
  - TP53 AmpliSeq Assay (IonTorrent)
- 5 patients : with low level a TP53 variant, subsequently went on to become TP53 positive by Sanger (post treatment),
- 1 patient : with low level a TP53 mutation passed away before subsequent Sanger testing.


Timeline of Clinical Progression between Diagnostic Patients
Patient
6
5
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (Years)
Diagnosed
Negative Sanger
Death
Negative sanger - with NGS SC
Positive sanger
Treatment


Time scale from a low level TP53 sub-clone to a detectable variant burden by Sanger sequencing.
Patient
6
5
4
3
2
1
0
0
1
2
3
4
5
Time from undetectable variant by Sanger (Years)
Negative sanger - with NGS SC
Positive sanger
Death

Conclusions

- NGS can detect lower level TP53 mutation burdens below the sensitivity of Sanger sequencing,
- Discovery means small TP53 sub-clones can be treated earlier with TKIs such as Ibrutinib, potentially prolonging survival.

Thank You

OXFORD Molecular Diagnostics Centre

Oxford University Hospitals NHS
NHS Foundation Trust

Acknowledgements

Helene Dreau
Dr Pavlos Antoniou
Dr David Bruce
Paula Ware
James Docker
Dr Anthony Cutts
Mohammad Ali
Dr Ruth Clifford
Adele Timbs
Dr Shirley Henderson
Professor Anna Schuh

http://www.oxford-translational-molecular-diagnostics.org.uk/

Platypus process