

Communications between Regional Diagnostic Centres and their Users

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Shared Aims

- Timely and Accurate Diagnosis of Haematological Malignancies through best use of available technology.
- Cost effective application of capital resources and professional expertise.
- Clear communication with Patients and Clinicians.

What should a user expect?

- A comprehensive test repertoire
 - Morphology and immunocytochemistry
 - Flow Cytometry
 - Molecular diagnostics
 - Cytogenetic Studies
- Clearly Defined Investigative Pathways
- Access to audit and quality assurance data
- Clinically appropriate and monitored reporting times
- Efficient transmission of reports and on-line/telephone help
- Published costs

What does the Central Laboratory need ?

- Correct and appropriate information about the patient
- Accurate and timely triage of specimens to be referred
- Specimens of suitable quality
- Prompt feedback and discussion if there is a problem.

Identifying specimens that may contain haematological malignancies How could this be improved?

- Making the best use of simple clues
 - Age
 - Site of disease
 - Previous medical history
- Every patient should have a blood count before biopsy
- Use the imaging data
 - Ultrasound
 - Pattern of Disease.

Pre-Diagnostic Tests for Lymphoid Malignancy to Guide Referral and Diagnostic Pathways

How many patients could be identified on a blood sample?

Full Blood Count

High sensitivity Flow Cytometry

Serum sample for protein analysis.

Simple molecular tests- t(14;18), MYD88

Cyclin D1 FISH or RQ-PCR

This will identify and diagnose most patients with:

CLL

Waldenstroms

Systemic Marginal Zone Lymphoma

Mantle cell lymphoma

About 50% of patients with follicular lymphoma.

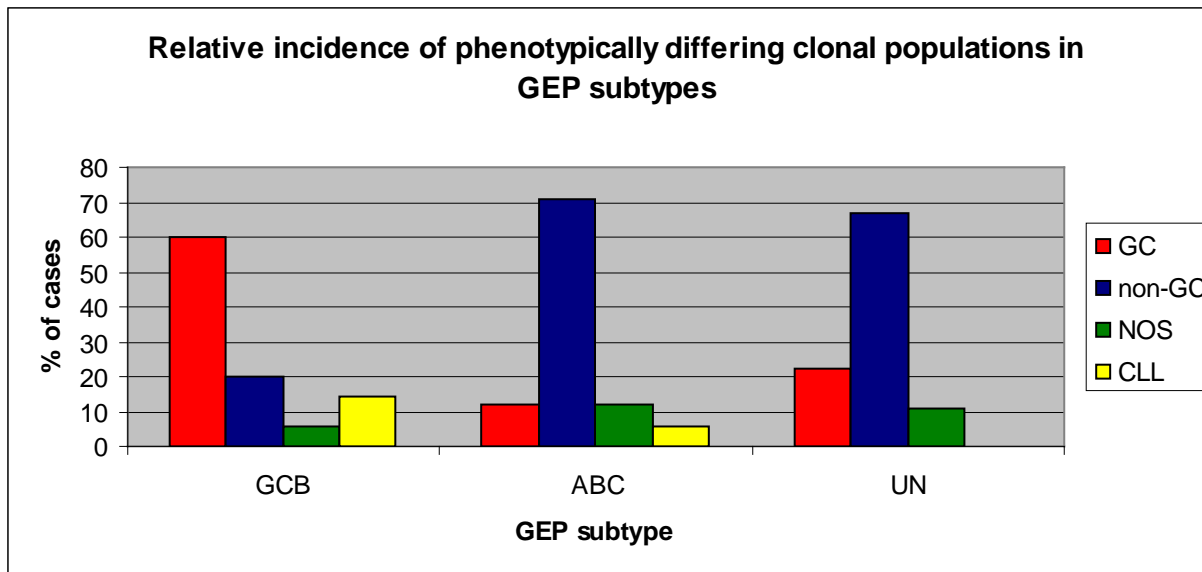
The decision to carry out further biopsies depends on clinical features.

What about DLBCL and Hodgkin Lymphoma ?

Most patients with DLBCL have a normal lymphocyte count but:

25% have a detectable clonal population

33% have severe B-cell lymphopenia.



Almost all CHL patients have elevated TARC and or sCD163

Pre-screening patients with peripheral blood examination

Pro and Cons

- Potentially very cost effective and quick
 - Test profile £110
 - Savings in out-patient and investigation costs
- Helps ensure prompt referral to the appropriate clinic
- Much higher levels of clinical judgement needed in some cases
 - Normal peripheral blood screen
 - Clinical features not consistent with peripheral blood findings.

Types of Specimen

An excised lymph node sent fresh to the laboratory remains the gold standard

- Best tissue processing and immunocytochemistry
- Flow Cytometry
- Best quality DNA and RNA.
- FISH on imprints

Requires care in handling and transport

Needle Core Biopsies

Would you have one?

- Very few properly designed comparative studies
 - Making a diagnosis is not the same as making a correct diagnosis with high confidence.
- Size is critical
 - A regional laboratory may need 25+ sections depending on the diagnostic complexity
- Sequencing and gene expression profiling are better than might be expected
- Diagnostic certainty is improved by a matching aspirate and peripheral blood sample
 - Flow cytometry and molecular diagnostics

Deciding on adequacy of small specimens is one of the most difficult challenges in haematopathology

Scientific and Political Changes will have a major impact on relationships between referral laboratories and their users

- The routine application of data rich high throughput technologies
 - Next generation sequencing
 - Gene expression profiling
 - Array based cytogenetics
- Stratified and Personalised Medicine
- How the NHS commissions services

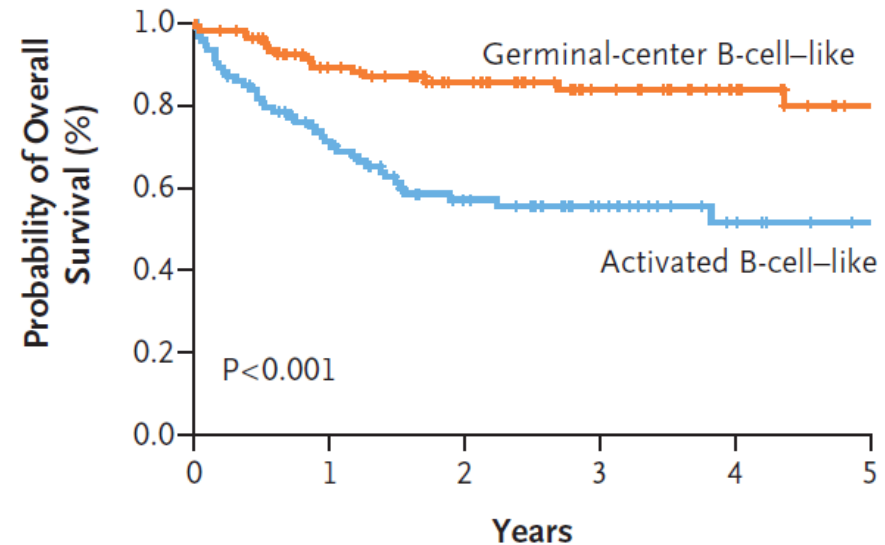
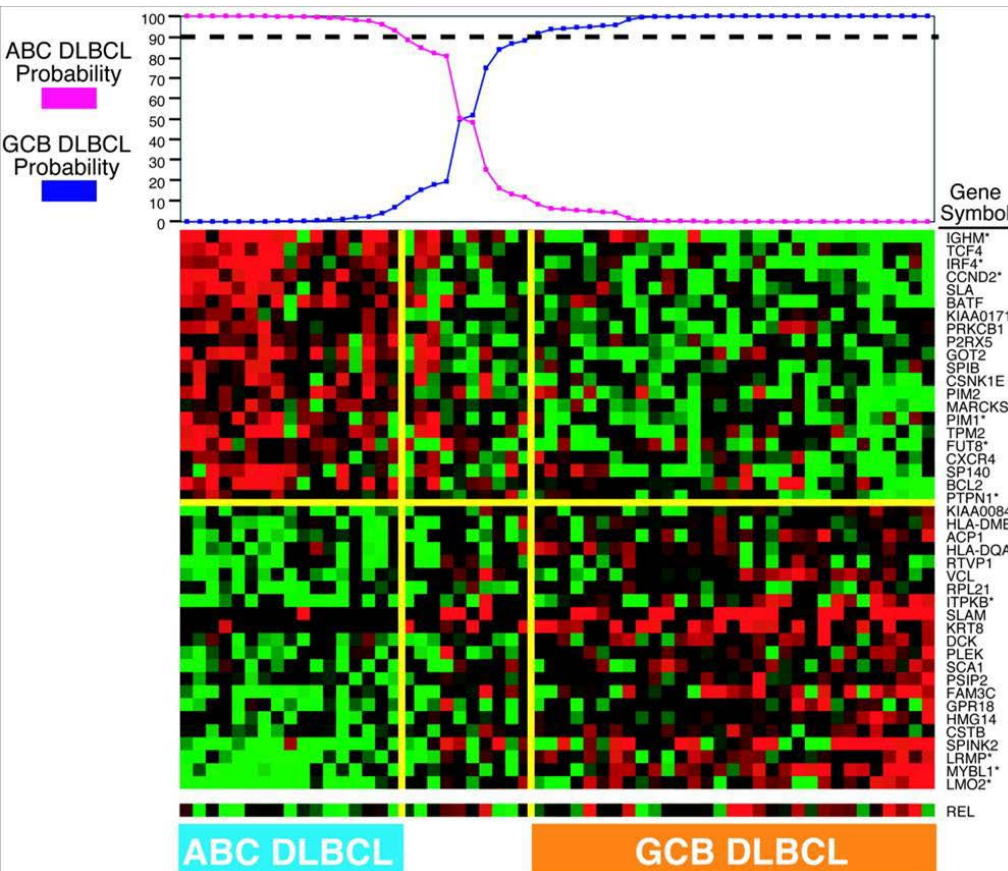
Data Flows and Effective Communications will be even more critical

Pathogenesis based classifications are central to the stratified and personalised medicine agenda

- Very large investments being made in introduction of molecular diagnosis of cancer
 - Haematological malignancies in the first wave.
- Next generation sequencing and gene expression profiling are affordable and deliverable in the routine laboratory
 - But a high throughput is required to balance cost and reporting times
 - Remarkably robust in FFPE specimens.
- Understanding the pathogenesis of haematological malignancy and the factors determining outcome is not complete but well advanced.

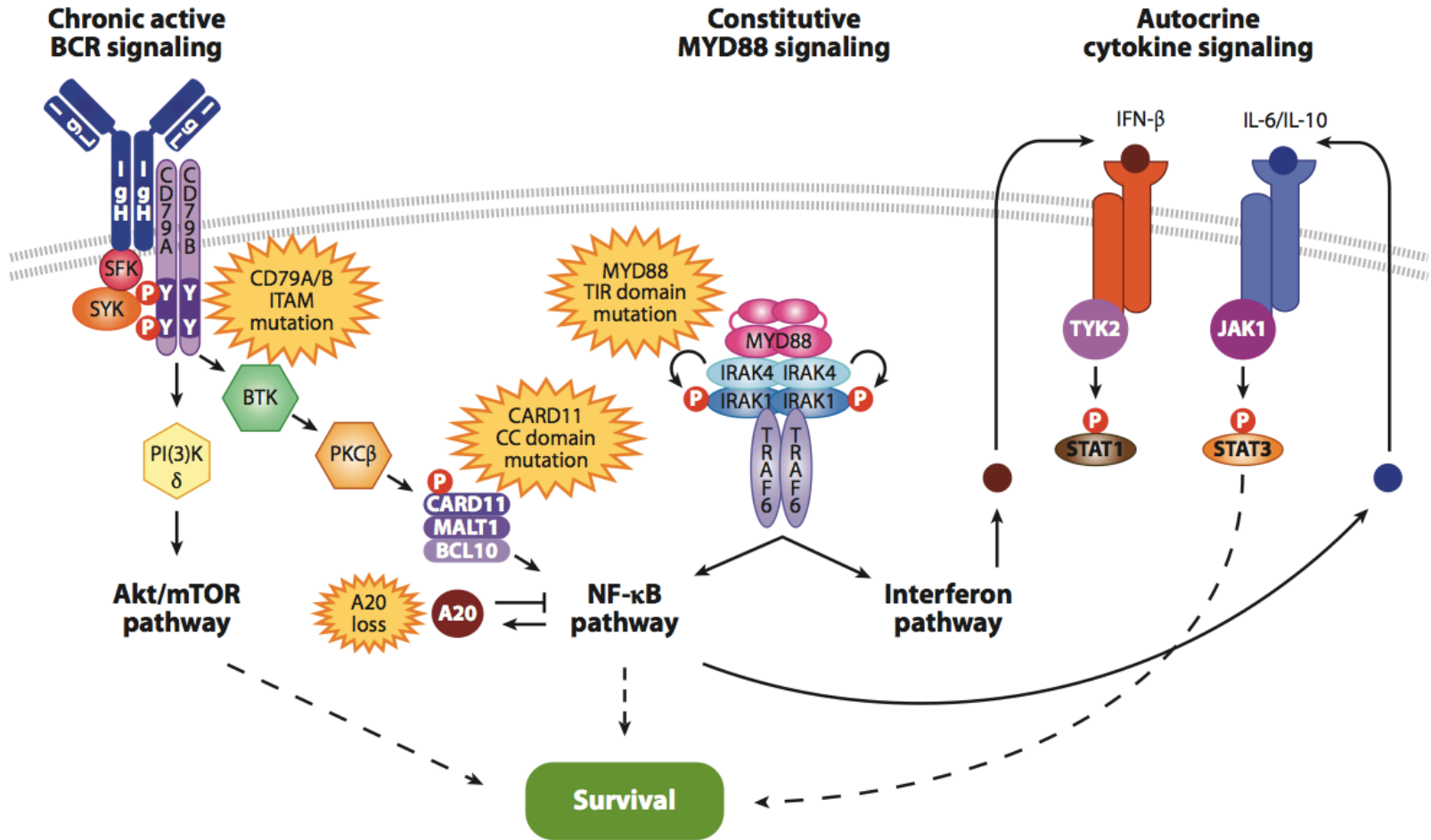
The Development of a Stratified Medicine Trial: from concept to delivery

- Wright algorithm
 - 27 gene predictor model to identify GCB and ABC subtypes of DLBCL
 - Prognostically relevant sub-classification, also re-validated in R-CHOP



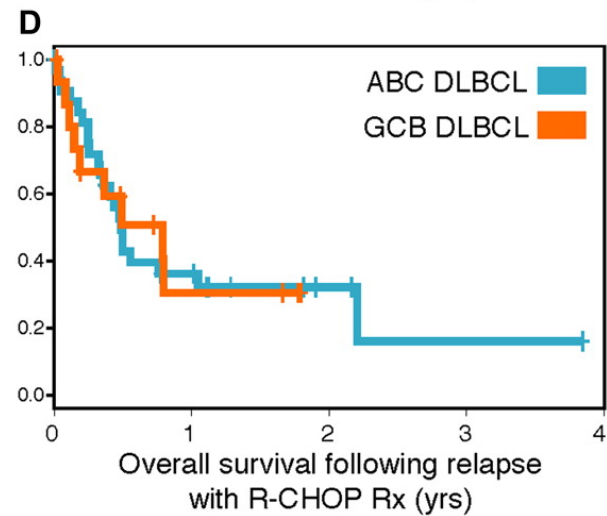
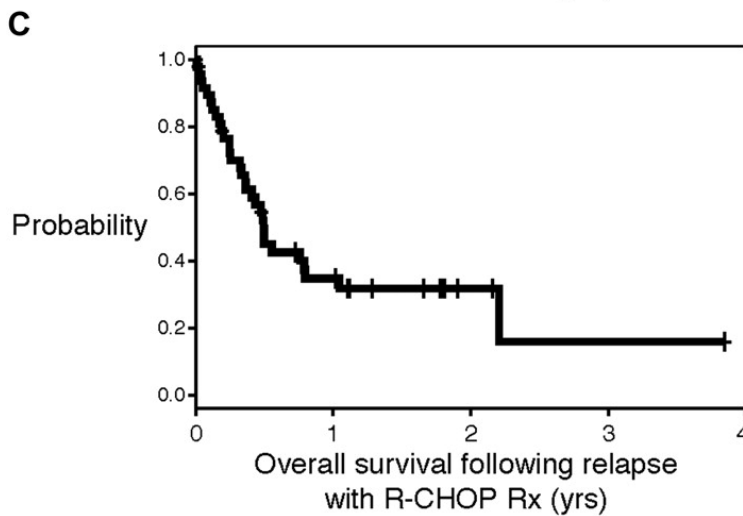
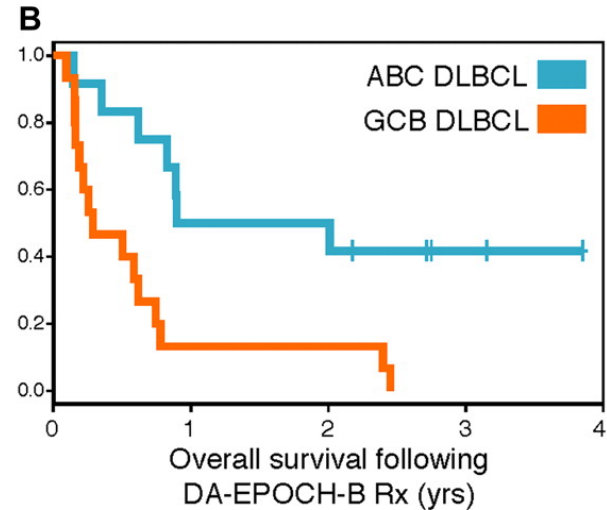
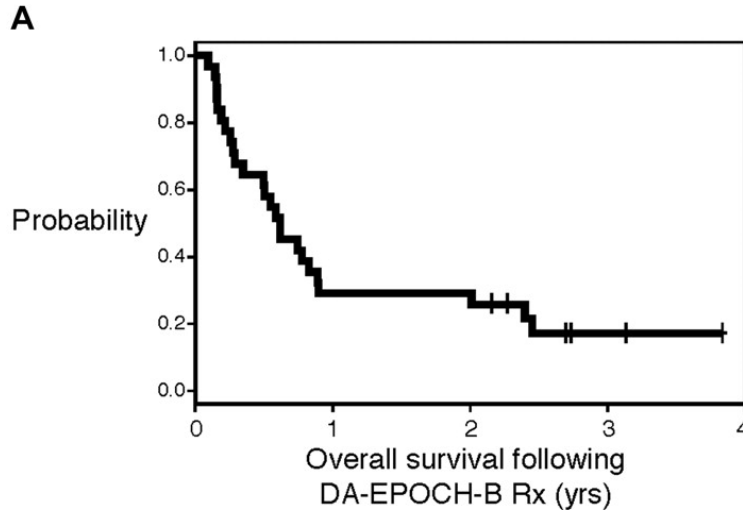
Lenz et al. NEJM 2008

The ABC Subtype relies on NFκB Pathway activation



Pathogenesis of Human B Cell Lymphomas*
 Arthur L. Shaffer III, Ryan M. Young,
 and Louis M. Staudt

Overall survival in patients with DLBCL. (A) Overall survival of 31 patients with de novo DLBCL who received DA-EPOCH-B.



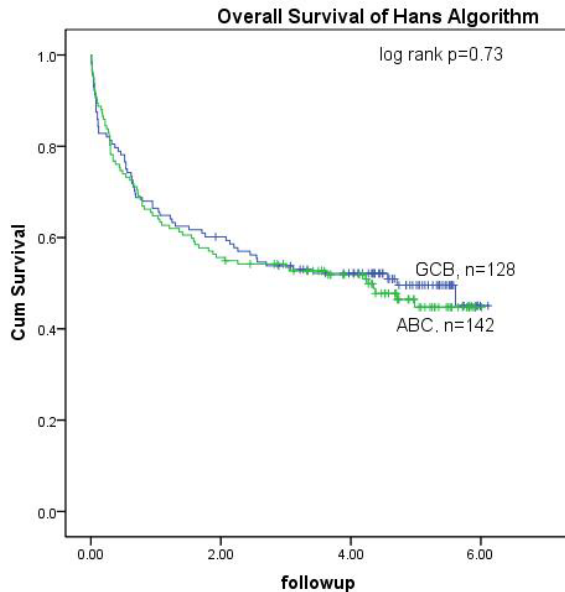
Dunleavy K et al. *Blood* 2009;113:6069-6076

Only Patients with an ABC type tumour should benefit from the addition of Bortezomib to standard therapy

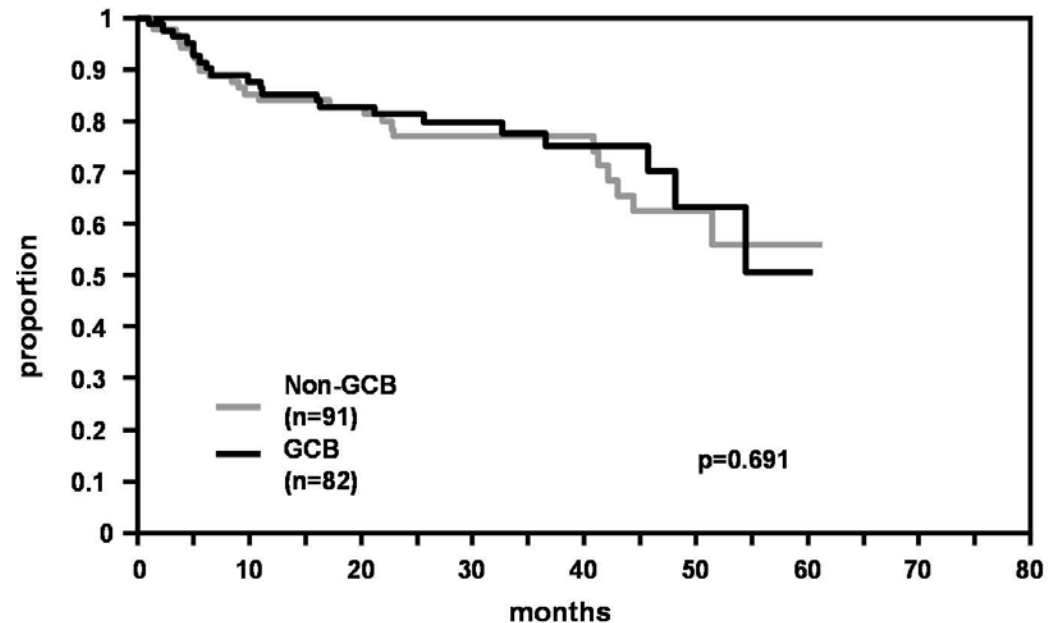
- Can patients be identified in 'real time' to allow randomization?
- How to design a trial to test the hypothesis?
- How can this be incorporated into the diagnostic pathway?

Could Immunocytochemistry be used in stratification?

The Hans Algorithm



HMDS Data



RICOVER 60 Trial

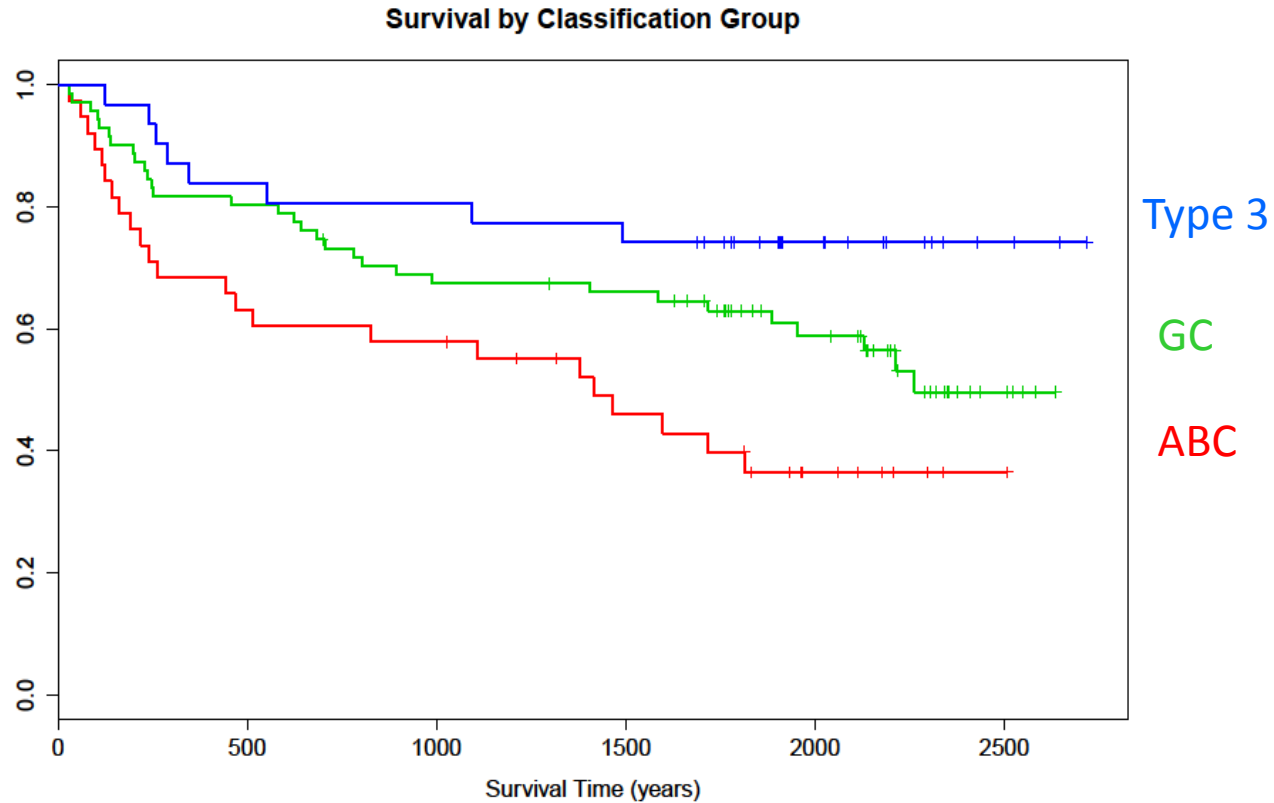
Immunocytochemistry is not suitable for treatment stratification.

Can whole genome expression profiling be carried out on FFPE

DASL Whole genome expression analysis

- **DASL** = cDNA-mediated **A**nnealing, **S**election, extension and **L**igation
- Gene expression of >24,000 transcripts.
 - Additional, unique probe sequences per gene increases the sensitivity of the assay
- Designed for degraded RNA, including FFPE
 - Use of random primers in addition to oligo-dT for cDNA synthesis, increasing efficiency in FFPE RNA
 - Probes span only 50 bases therefore suitable for degraded RNA
- Generation of expression profiles from as little as 100-200ng FFPE RNA

Can whole Genome Expression Profiling be used? ABC/GCB/Type 3 classification is highly reliable on FFPE derived RNA



Designing a real time classifying algorithm (the hardest bit)

PLOS ONE: A Microarray Platform-Independent Classification Tool for...ative Analysis of Gene Expression in Diffuse Large B-cell Lymphoma

15/06/2013 12:00

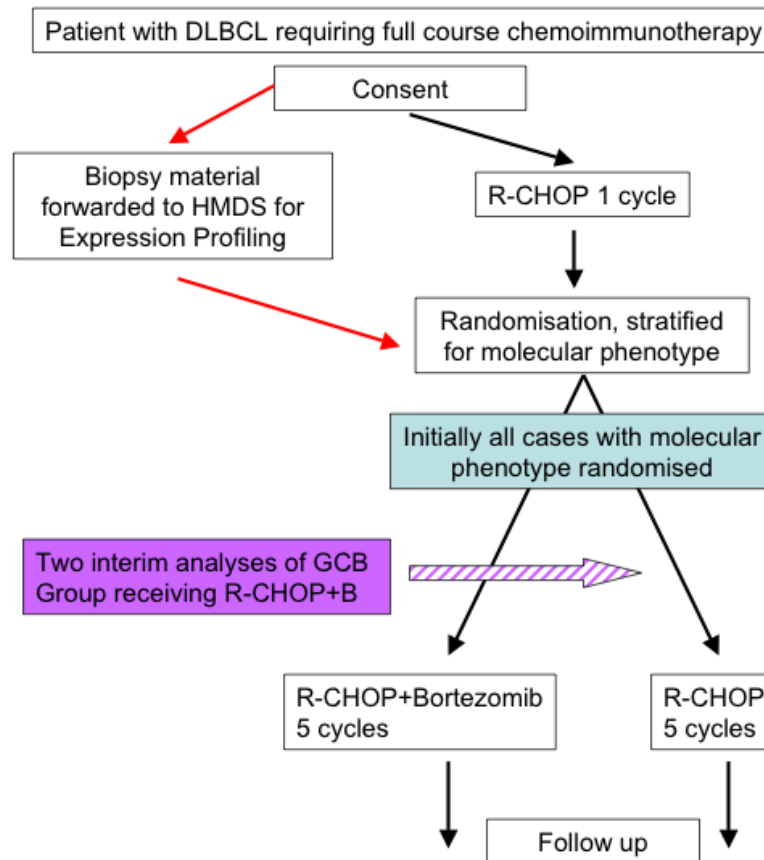


A Microarray Platform-Independent Classification Tool for Cell of Origin Class Allows Comparative Analysis of Gene Expression in Diffuse Large B-cell Lymphoma

Matthew A. Care, Sharon Barrans, Lisa Worrillow, Andrew Jack, David R. Westhead, Reuben M. Tooze

Designing and implementing the trial

Trial Outline:



REMoDL-B

What platforms should we invest in: Will sequencing be the only diagnostic technique for lymphoma?

	MYC – Rearranged	MYC-non rearranged
MYC expression signature	89	83
No MYC signature	20	514

The Pathogenic pathway is not characterised by genotype alone

- Additional factors such as epigenetics, miRNA and stroma may effect phenotype and clinical behaviour
- Pathway analysis from NGS and GEP data is an emerging area of bioinformatics

Aim to have linked sequence, GEP and clinical data on 1000 DLBCL in 2014 and 3000 by 2016.

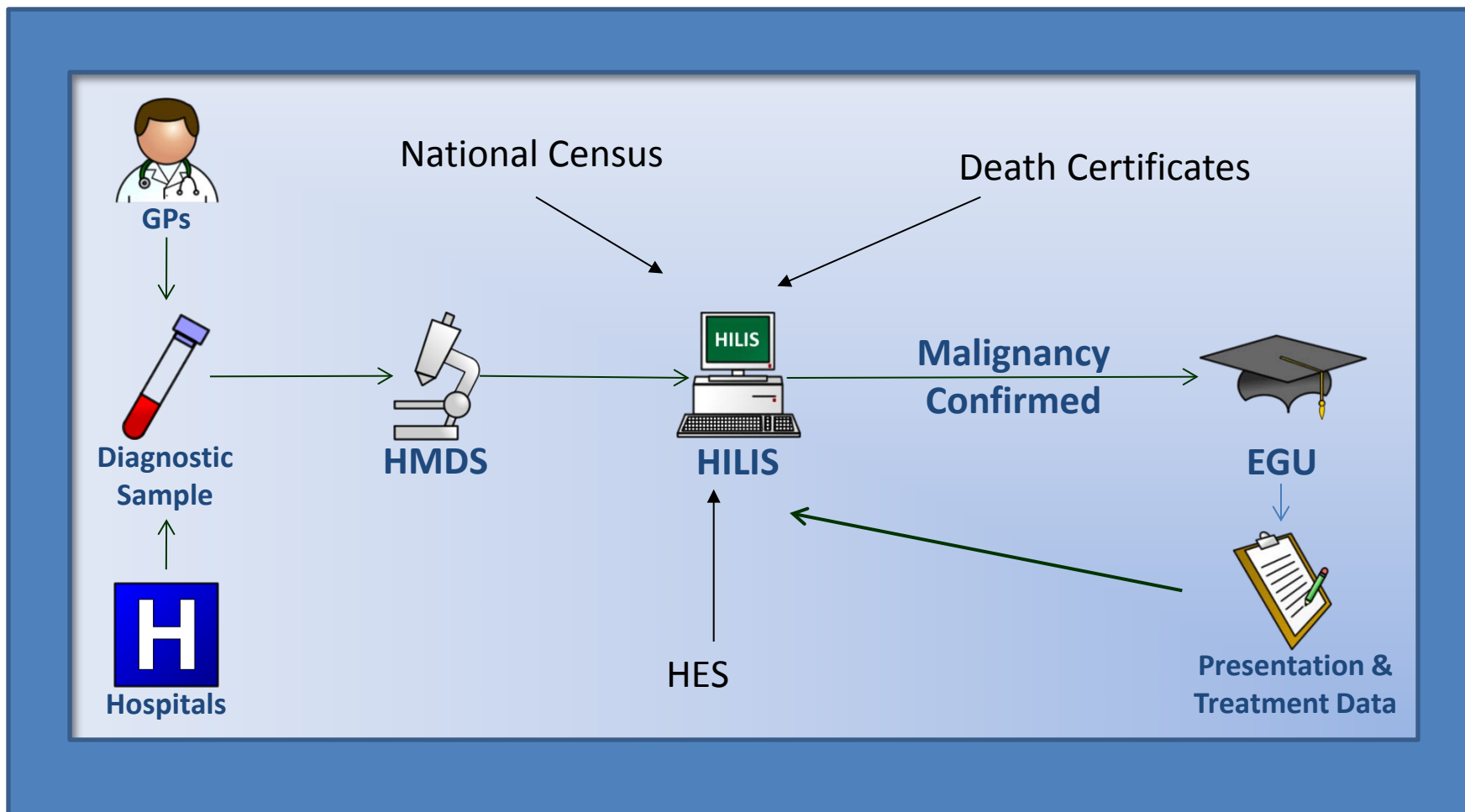
Linkage to clinical data is the hidden ingredient that makes it all work

Optimal utilisation of new diagnostic technologies require:

- Integration of clinical, laboratory and outcome data
 - Prognostic models
 - Continuous adjustment to novel therapies
- New approaches to disease classification.
- Effective means of presenting the data to clinicians and patients.

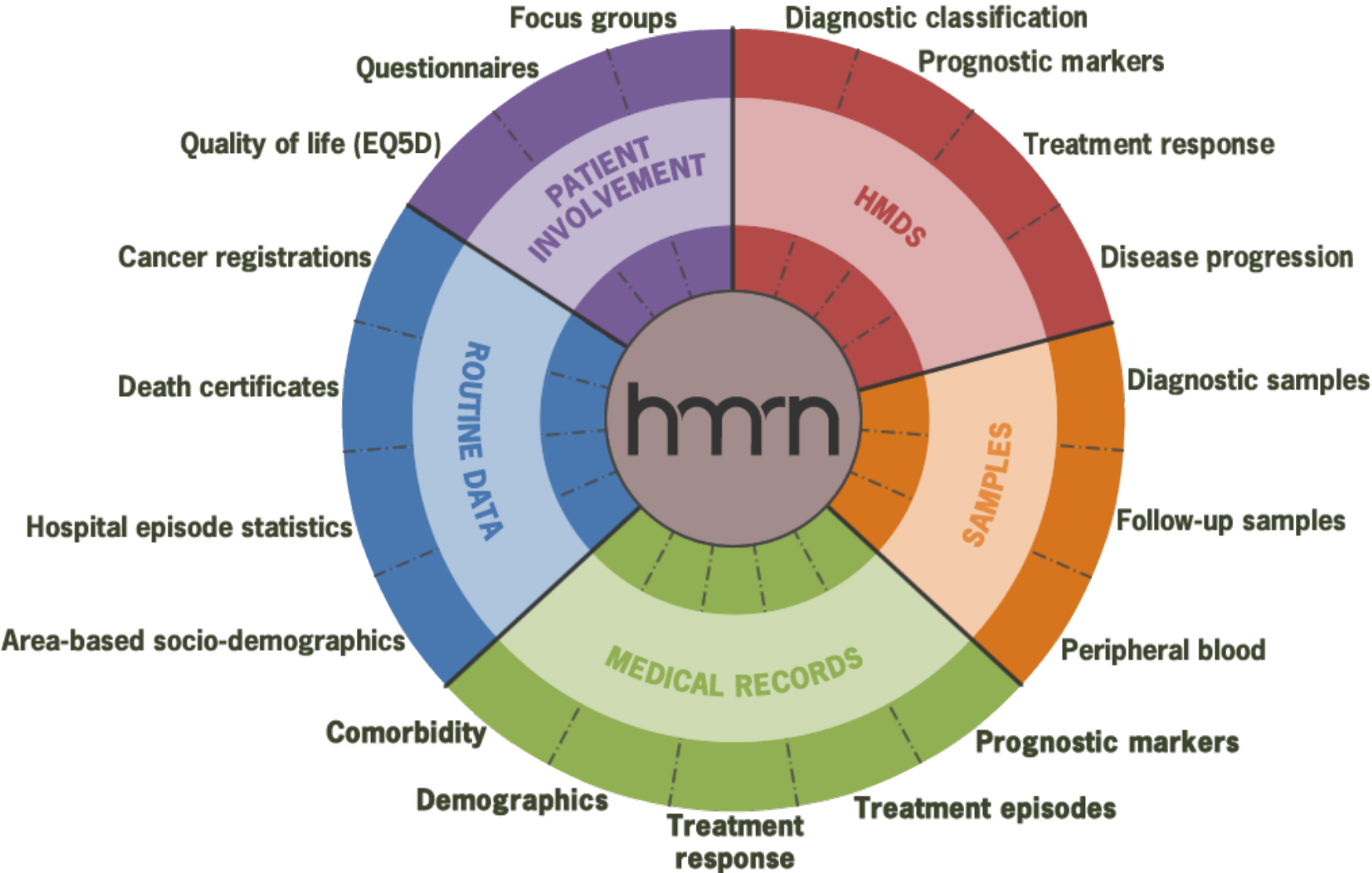
How can local data be used to inform centrally performed diagnostics

Linking Laboratory and Clinical Data HMRN in Yorkshire and the Humber A population based approach



Ascertainment is >99%

HMRN - Data



Sequential diagnostic and treatment data: a neglected area

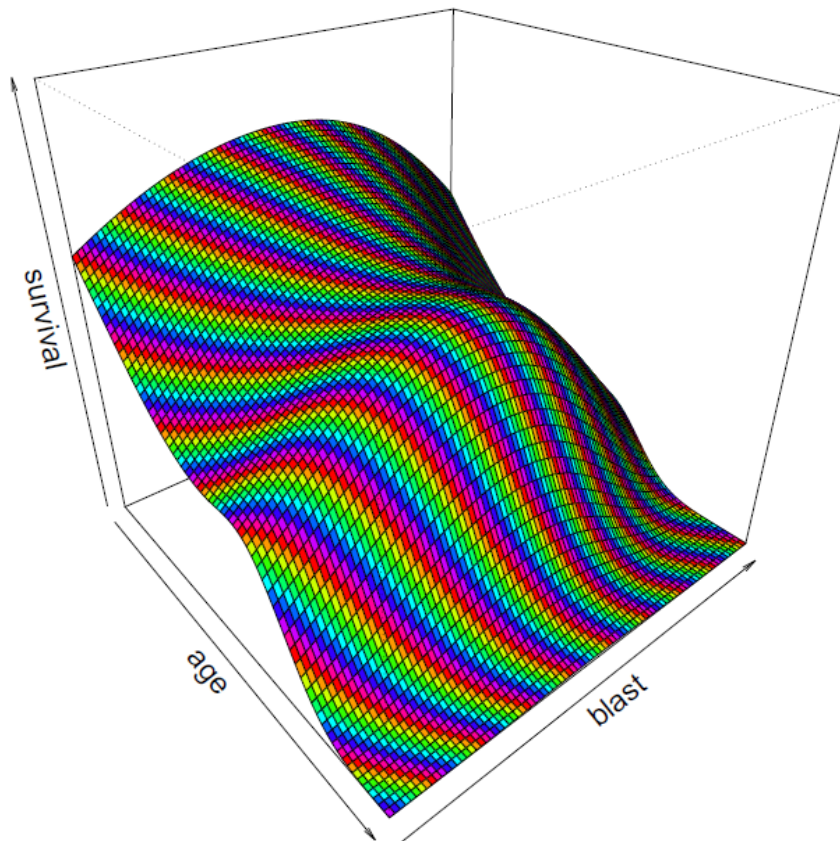


CVP/Rituximab: Rituximab + Cyclophosphamide + Vincristine + Prednisolone
 CHOP: Cyclophosphamide + Vincristine + Prednisolone
 FC/Rituximab: Rituximab + Fludarabine + Cyclophosphamide
 CHOP/Rituximab: Rituximab + Cyclophosphamide + Doxorubicin + Vincristine
 ESHAP/R: Rituximab + Etoposide + Methylprednisolone + Cytarabine + Cisplatin
 FC: Fludarabine + Cyclophosphamide
 Mini-BEAM: Carmustine + Etoposide + Cytarabine + Melphalan
 DHAP/Rituximab: Carmustine + Etoposide + Cytarabine + Melphalan

Follicular lymphoma – watch & wait

Database integration allows the development of Prognostic models and clinical decision aids

Which Patients can benefit from Intensive Treatment?



Modelling patient outcome on intensive and non-intensive therapies using flow cytometric blast counts.

Passive data collection through Database Linkage

The image shows a screenshot of a web browser displaying the Flatiron Health website. The browser's address bar shows the URL www.flatiron.com. The website's navigation menu includes links for **FLATIRON**, **PROVIDERS**, **LIFE SCIENCES**, **CAREERS**, **ABOUT**, and **BLOG**. The main heading is **Introducing OncologyCloud™**, with the subtext: "The oncology industry's first cloud-based data platform and the new standard for real-time insights and intelligence." Below this, there are two buttons: "For Providers" and "For Life Sciences", both with right-pointing arrows. At the bottom of the page, there is a preview of the OncologyCloud interface. This interface shows a dashboard for a user named "Dr. Michael" with sections for "Alerts", "Status", "Key Metrics", and "Patient Volume by Cancer Type". The "Patient Volume by Cancer Type" section includes a line chart and a table with the following data:

Cancer Type	2012	2013	2014	2015	2016	2017	2018	2019	2020
Breast	247	275	312	351	391	431	471	511	551
Prostate	215	232	250	268	286	304	322	340	358
Lung	187	198	209	220	231	242	253	264	275
Colorectal	155	166	177	188	199	210	221	232	243
Total	584	670	748	826	904	982	1060	1138	1216

Google paid \$130M for this company in 2014

Realising Patient Benefit by Linking Diagnostic and Data

Providing analytical tools to the end user rather than 'diagnosis'

- Highly effective prognostic models linked to patient decision aids
- Targeted therapies
- Economic stratification

These tools can make local service delivery more rather than less challenging.

Diagnostic Categories

Do we really need them at all?

- Diagnostic Categories place artificial and often highly misleading constraints on Clinical Decision Making.
- Newly presenting patients can be matched across the whole data base for similarity
 - Machine learning tools
 - Not simple

Similarity Analysis can be used to compare the outcome of different treatment.

Correlation based similarity search in the Hummel dataset with query case 31 (green) and the most similar other cases based on expression of 24 genes

Case number	Classification	Genetic group	Treatment regime	Survival status
31	Intermediate	MYC.complex	???	Alive
139	Intermediate	MYC.neg	CHOP-like	Dead
46	Intermediate	MYC.simple	Intensive	Alive
40	Intermediate	MYC.complex	CHOP-like	Dead
100	Intermediate	MYC.simple	Intensive	Alive
24	mBL	MYC.complex	CHOP-like	Alive
64	mBL	MYC.neg	Intensive	Alive
218	Intermediate	MYC.neg	CHOP-like	Dead

How will these services be delivered in the next 5 years?

- Organised local networks are breaking down and will probably disappear – no longer supported by the NHS in any meaningful way
 - There will be a competitive market
- Will haematological malignancy diagnosis exist separately from biomedical hubs?
 - The NHS will commission about 10-12 hubs in 2014.
- Investment is needed (and is available)
 - The main providers will be NHS/private organisations.
- Will there be re-convergence of diagnostic strategies for all cancer sites?


Conclusions

- Good Communication between central and local services are critical and becoming more important
- There is scope for improvement in the way specimens are handled.
- The future organisation of services is largely unknown and will be more market driven
- The next generation of diagnostic has great promise to improve patient care but will crucially depend on efficient data flows.
- New analytical tools will be very challenging for local service delivery and will require new skills and attitudes



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Eve Roman, (ECSG, York)
Data collection & Statistical analysis

Haematologists of YHHN
- Clinical data



YHHN



Peter Johnson
Andy Davies
REMoDL-B



Beating Blood Cancers