

Tumor Genomics: Biology and Implications for Cellular Therapies

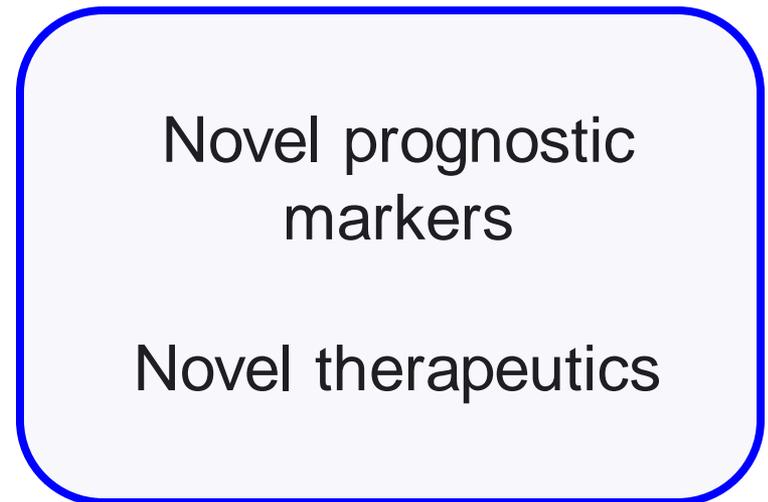
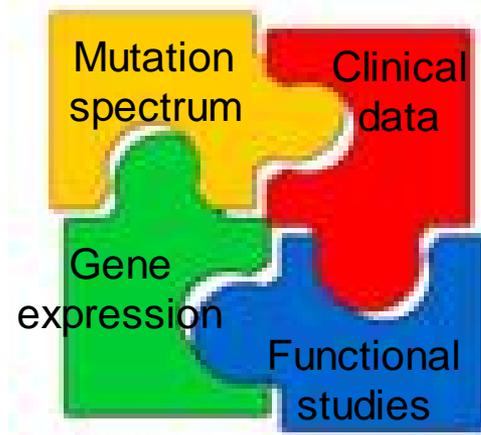
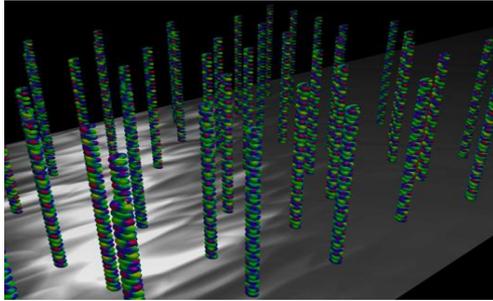
Catherine J. Wu, MD

Cancer Vaccine Center

Dana-Farber Cancer Institute, Boston, MA

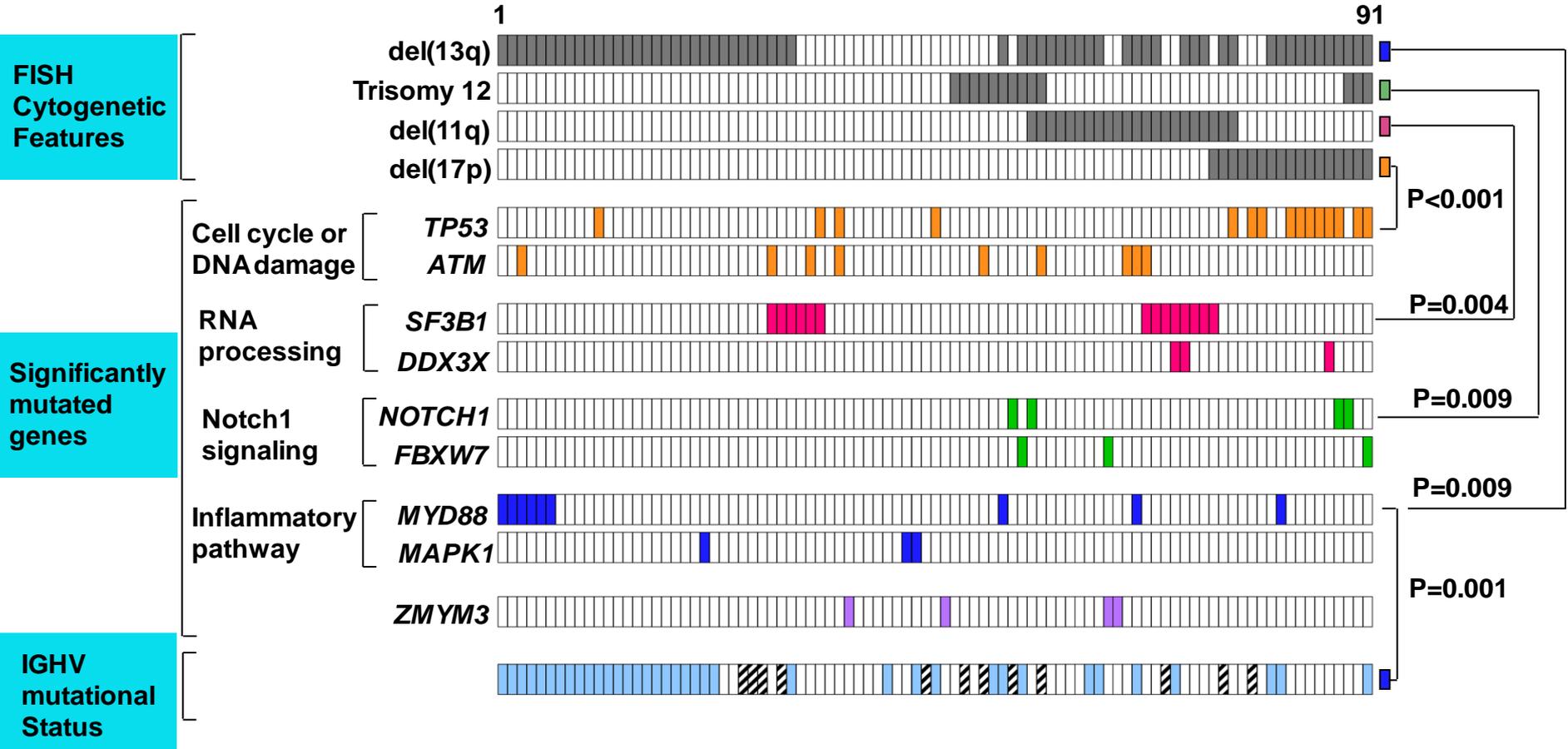


Challenge: understanding the basis of clinical heterogeneity within a malignancy



Ley TJ *NEJM* 2010; Chapman *Nature* 2011; Morin RD *Nature* 2011; Tiacci *NEJM* 2011; Puente *Nature* 2011; Treon *NEJM* 2012

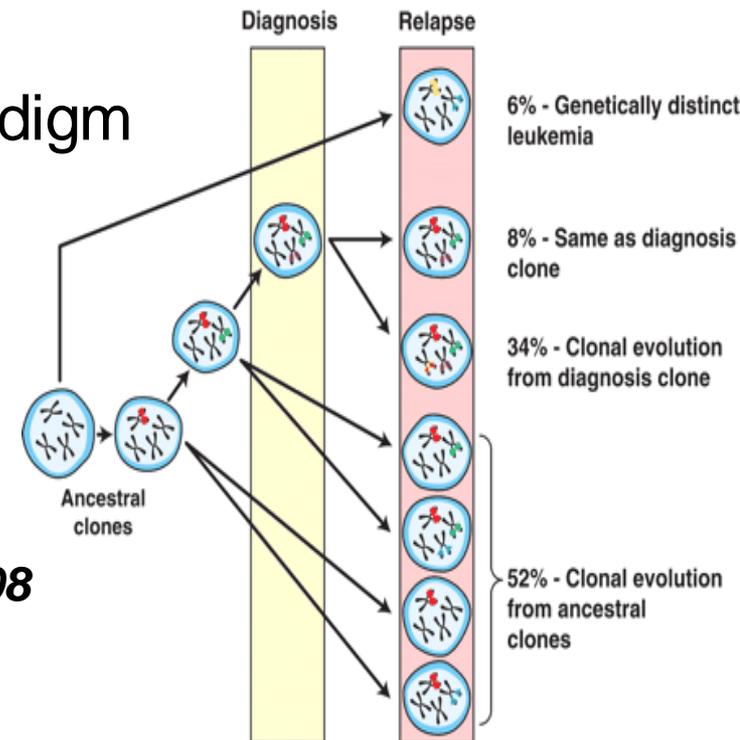
DNA sequencing reveals genetic subgroups of CLL



Intratumoral heterogeneity and clonal evolution

- Existence of tumor subpopulations has long been theorized
 - Genetic instability expected to lead to heterogeneity as the neoplasm progresses, resulting in diverse and genetically distinct subpopulations within the tumor (*Armitage & Doll Br J Cancer 1954; Nowell, Science 1976*)

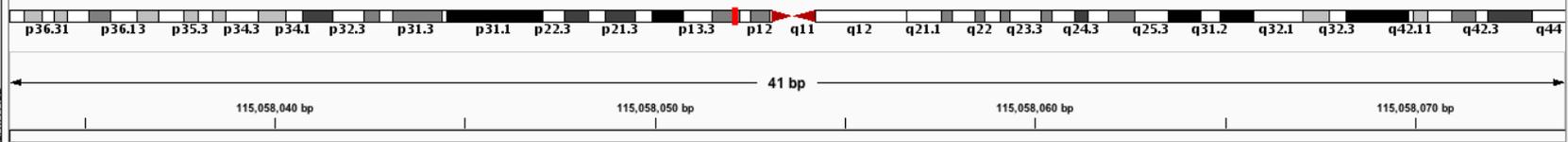
- Existing evidence supports this paradigm
 - Cytogenetics
 - FISH
 - SNP



Mullighan Science 2008
Keats Blood 2012

WGS for dissection of subclonal architecture

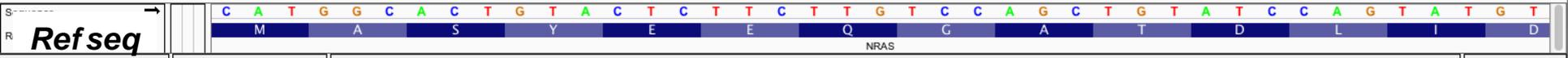
- Whole genome sequencing
 - Relatively low coverage (30x)
 - Can detect thousands of mutations per sample
 - A spectrum of allelic frequencies across genetic alterations is typically observed
- Clusters of genetic alterations can be identified
 - Mutations present in the **all** cell fractions: consistent with a **cancer-initiating** population
 - Mutations present in a **subset**: consistent with **a later subpopulation**
- Across hematologic malignancies, a high degree of clonal heterogeneity observed, with marked changes in genetic make up at relapse



NAME
DATA TYPE
DATA FILE



**Tumor
seq
reads**

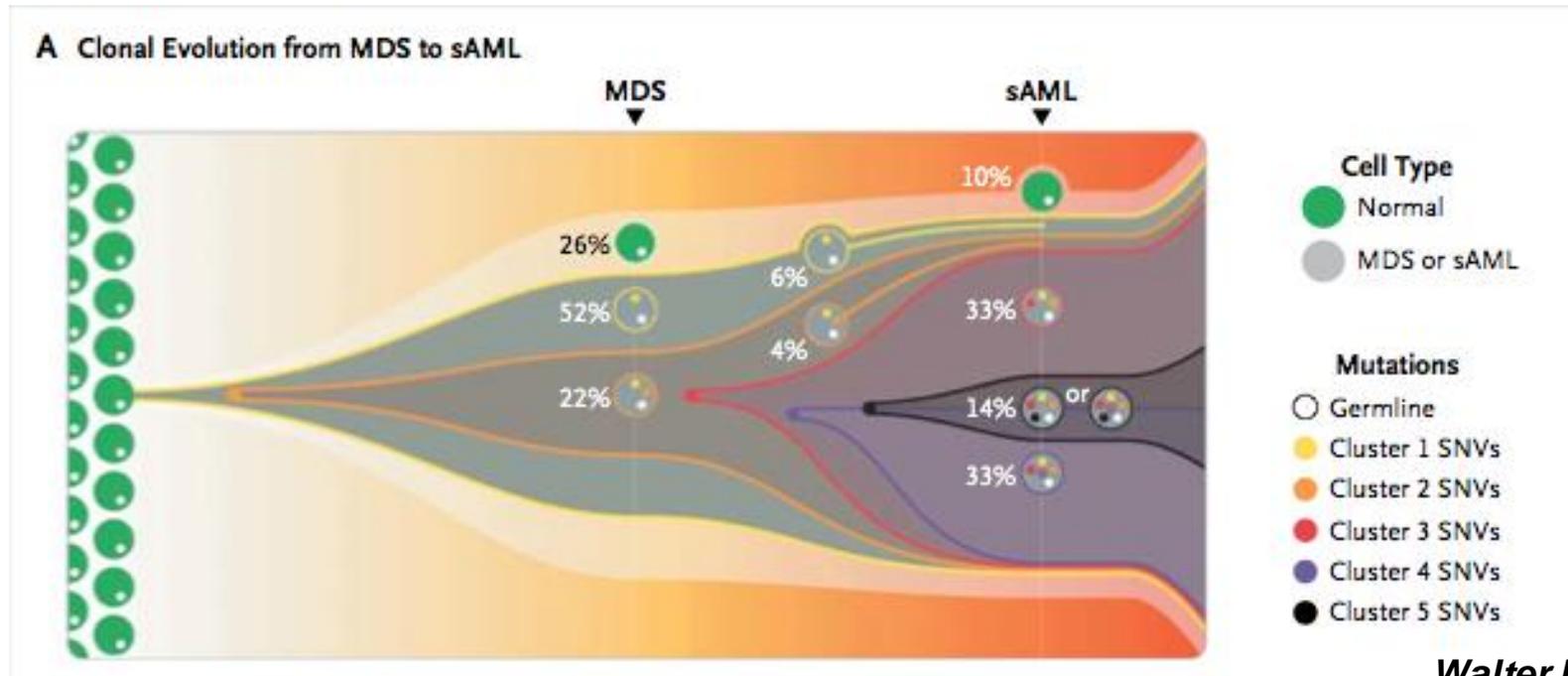


WGS for dissection of subclonal architecture

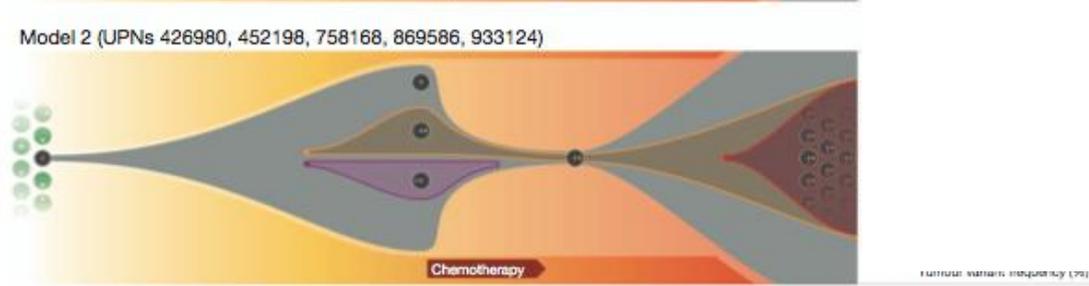
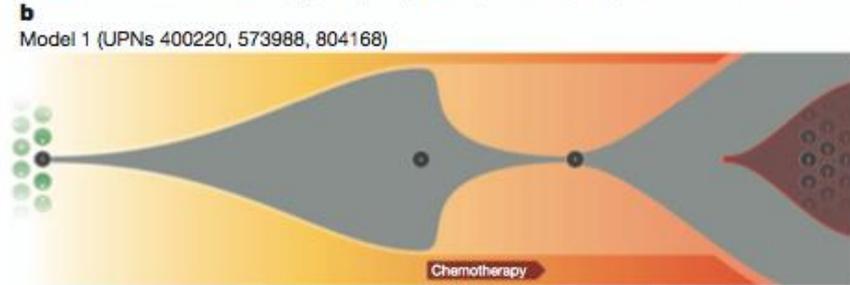
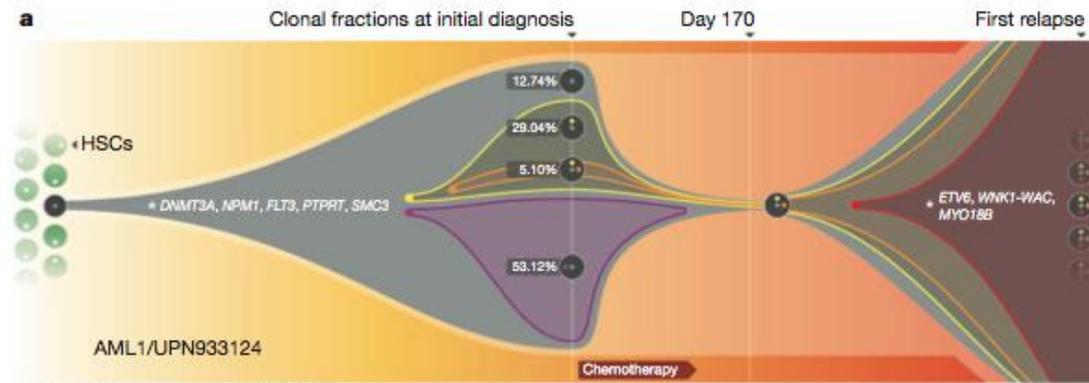
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Clonal evolution in MDS → secondary AML

- WGS of paired skin and marrow from 7 pts with sAML
 - Antecedent MDS BM genotyped
- Progression to acute leukemia was defined by persistence of an antecedent founding clone, with 182-660 somatic mutations, with the outgrowth or emergence of at least one subclone
- Supports model of sequential development of cancer

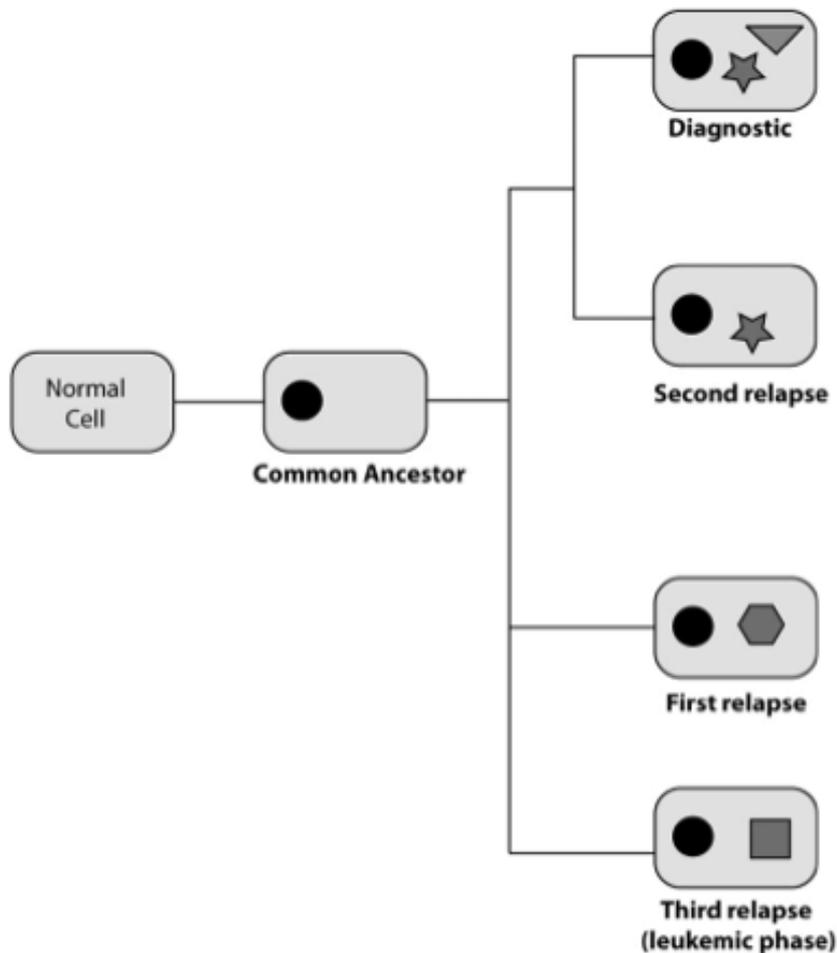


Clonal evolution in AML



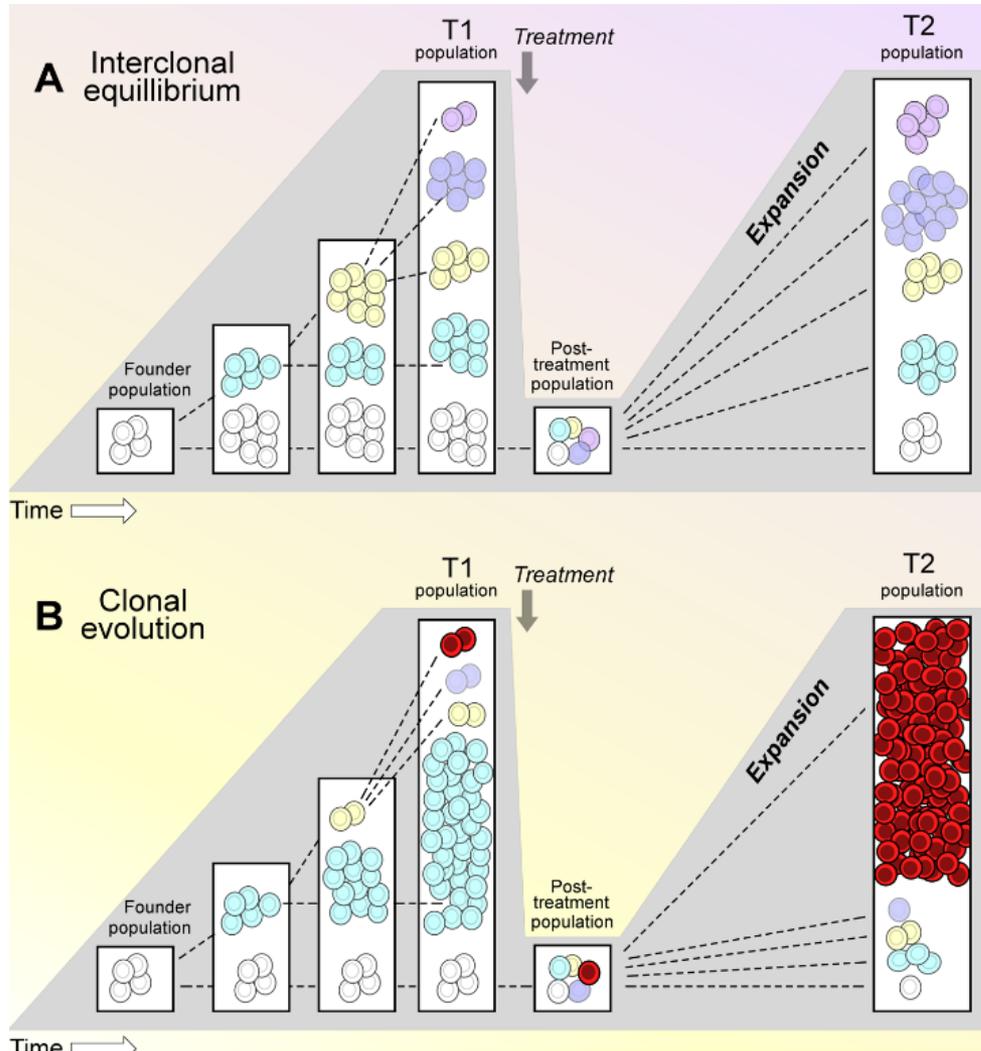
- WGS of 8 paired and relapse samples
- All acquired new mutations at relapse
- Two major patterns of relapse:
 - founding clone in 1^o tumor gained mutations and evolved into the relapsed clone
 - Subclone from founding clone survived initial therapy, gained additional mutations and expanded at relapse
- Possibility that remission-inducing chemotherapy shapes genetic evolution

Clonal evolution in MM



- WGS of 1 case of MM, across 4 timepoints over a 5 year period
 - From dx, 2 relapses and transition to plasma cell leukemia
 - t(4;14)
- Tumor heterogeneity at dx
- Multiple independent yet related clones at dx that rose and fell in dominance

Clonal evolution in CLL



- WGS of 3 pts with unmutated *IGHV*
- Sampled 5 times for up to 7 yrs
 - before and after Rx: chlorambucil, fludarabine, rituxan, ofatumumab
- Diverse patterns
 - Interclonal equilibrium
 - Clonal evolution

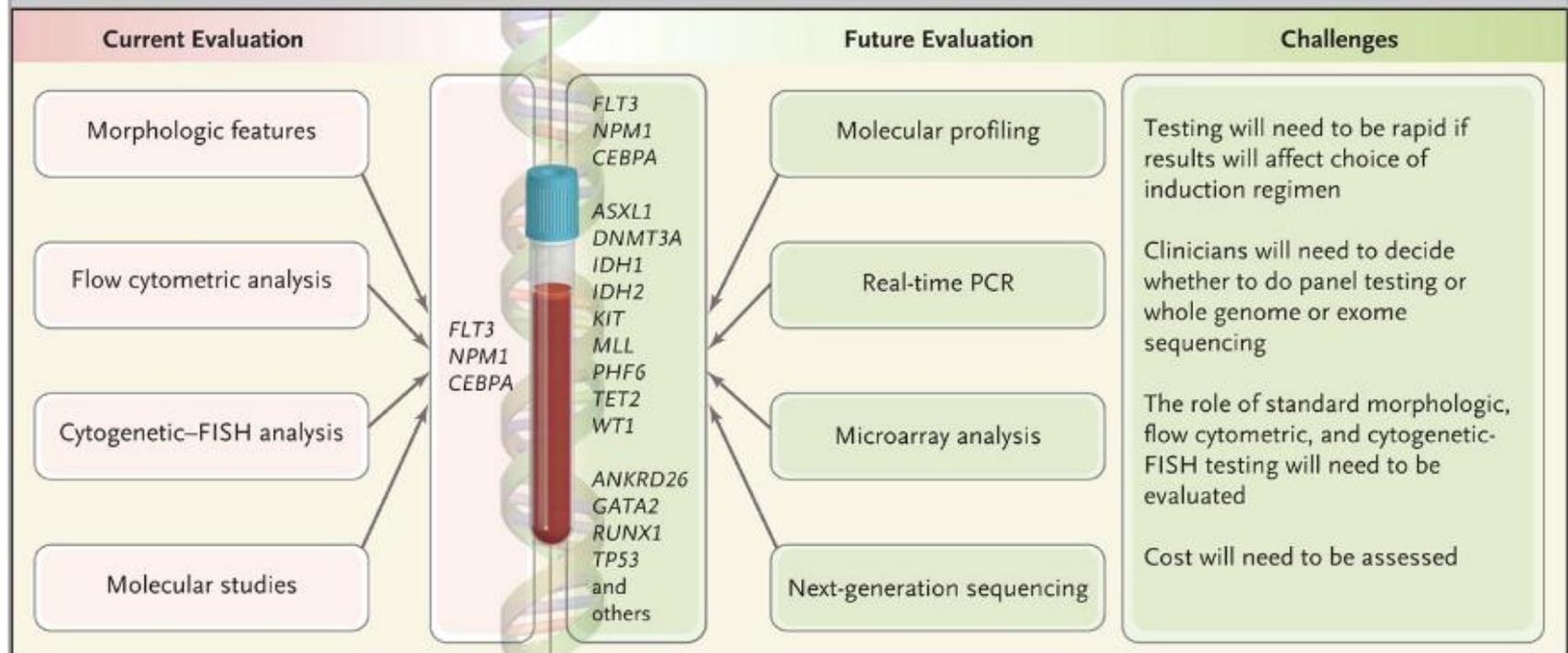
Summary and conclusions from these studies

- Similarities between malignant blood diseases but also differences
 - Acute leukemia: all with clonal evolution
 - Indolent B cell malignancy: heterogeneous patterns of clonal evolution
- Branched rather than linear evolution observed
 - Implies that genetic evolution results from complex fitness equilibrium of highly diverse populations
- Co-existing subclonal populations likely harbor driver lesions that are expected to provide fitness advantage
 - ?growth of subclones is limited by mutual competition (clonal interference)

Do subclonal populations matter?

- Studies of CNVs and WGS: Relapsed clones can be traced to pretreatment minor clones
 - Degree of genetic heterogeneity can be an important determinant of therapeutic outcome
- Questions:
 - How frequently are the patterns of interclonal equilibrium or of clonal evolution observed in patients; how do they relate to known prognostic risk factors?
 - What are the underlying genetic factors that determine whether subclones recede or expand?
 - Do presence of subclonal populations have clinical impact?
 - Does presence of clonal evolution hold prognostic or predictive significance?
 - Can identification of subclonal mutations prior to therapy anticipate the composition of the relapsing tumor?

So many opportunities, so many challenges....



Does consideration of subclonal populations fit into diagnostic schemes as well?

Potential therapeutic challenges presented by intratumoral heterogeneity

- An argument in favor of adopting combination rather than single-agent sequential therapies
 - Eradicate dominant and minor clones
- Avoid suboptimal therapies that eradicate sensitive clones, that could cause “competitive release”
 - Would allow resistance (fitter) clones to predominate
- ?Efficacy of highly targeted therapeutic approaches
- Selective pressures exerted by DNA damaging agents
- Potential retained sensitivity to previously administered chemotherapy

The promise of immunotherapy

Science MAAS

The Clonal Evolution of Tumor Cell Populations

Acquired genetic lability permits stepwise selection of variant sublines and underlies tumor progression.

Peter C. Nowell

“One may ultimately have to consider each advanced malignancy as an individual therapeutic problem....**Immunotherapy becomes a leading candidate** for the easiest means of destroying the remainder of the neoplastic clone...it is more feasible to produce specific cytotoxic antiserums or lymphocytes against a particular tumor than to design a specific chemotherapeutic agent for each neoplasm.”

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