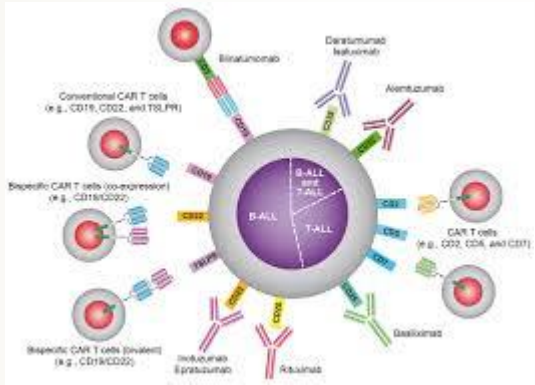
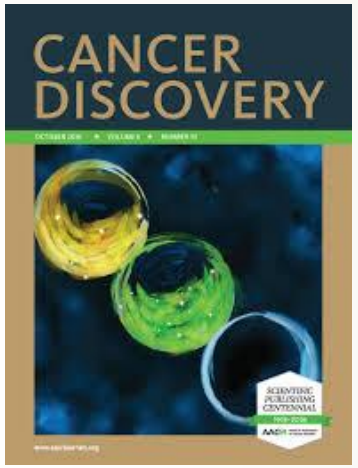
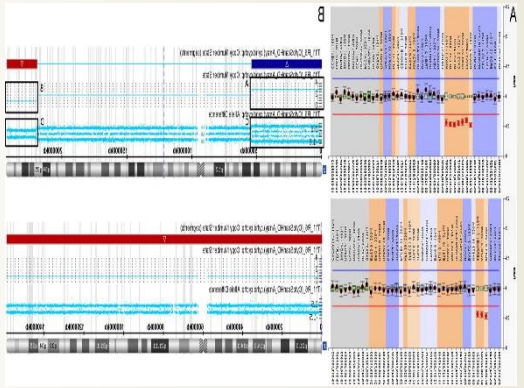
Present Genetic Landscape of Pediatric BCP-ALL in Iran and future directions for Risk Stratification







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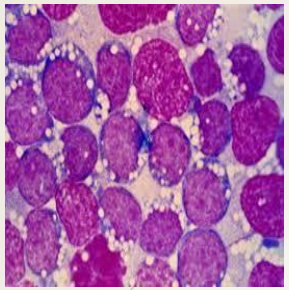
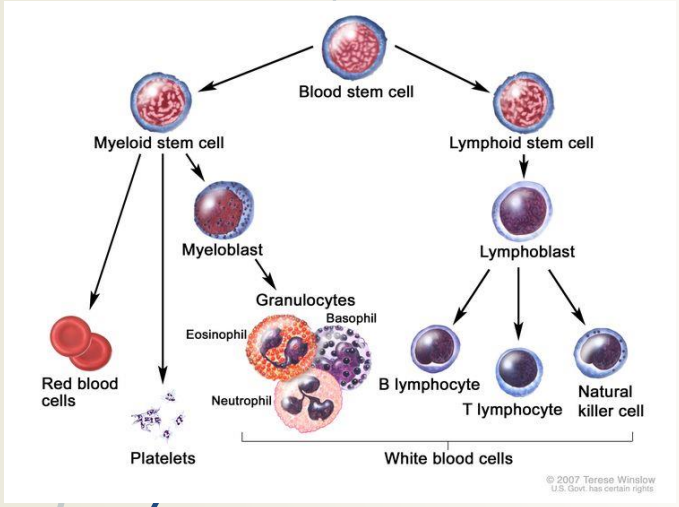
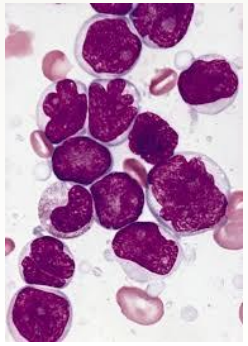
Morphology and immunophenotypic classification

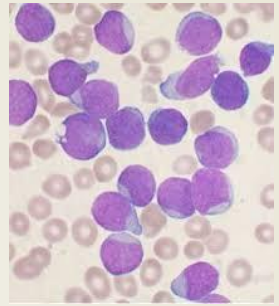






Morphological subtypes (FAB)

	L1	L2	L3 Burkitt's
Morphology	Homogenous	Heterogeneous	Homogenous
Size	Small	Variable	Small
Cytoplasm	Little	More	Vaculated
Nucleoli	Not prominent	Prominent	Prominent
Genetics	Variable	Variable	t(8;14) cmyc



Immunophenotypic Spectrum of ALL

Classification	Immunophenotype
Precursor B-cell ALL	
▪ Pro-B ALL*	CD10-, CD19+, CD79a+, TdT+
▪ Pre-B ALL†	CD10+, CD19+, CD22+, CD79a+
Mature B-cell ALL	Surface Ig+, lambda or kappa light chains, TdT-, CD20+/-
T-cell ALL	Cytoplasmic or surface CD3, variable expression of CD1a, CD2, CD5, CD7, TdT, CD52

*Early precursor B-ALL.
†Previously called common B-cell ALL.

NCCN. Clinical practice guidelines in oncology: acute lymphoblastic leukemia. v.2.2015.

Slide credit: clinicaloptions.com

Hallmarks of Cancer

- **Somatic genetic alterations** that initiate and drive carcinogenesis are the hallmarks of cancer.
- Genomic profiling has revolutionized our understanding of cancer and refined the classification of patients into clinically relevant subgroups.
- In pediatric B-cell precursor (BCP-ALL) significant chromosomal abnormalities provides
- Important diagnostic and prognostic information that is routinely used in risk stratification for treatment.

Recent information about abnormalities in B-Cell development signaling pathways

- Cell cycle regulation
- Transcriptional activation
- Tumor suppression
- Apoptosis
- Drug responsiveness

WHO classification B-cell lymphoblastic/lymphoma classification

- leukemia/lymphoma, not otherwise specified(NOS) B-cell lymphoblastic
- leukemia/lymphoma, with recurrent genetic abnormalities
- B-cell lymphoblastic leukemia/lymphoma with **hypodiploidy:**

WHO classification B-cell lymphoblastic/lymphoma classification

- B-cell lymphoblastic leukemia/lymphoma with
- t(v;11q23)[MLL rearranged]

- B-cell lymphoblastic leukemia/lymphoma with
- t(12;21)(p13;q22)[ETV6-RUNX1]

- B-cell lymphoblastic leukemia/lymphoma with
- t(1;19)(q23;p13.3)[TCF3-PBX1]

WHO classification B-cell lymphoblastic/lymphoma classification

- **Near haploid (24 to 30 chr)** somatic alterations targeting receptor tyrosine kinase and RAS signaling), in particular involving *NF1*), histone modifiers mainly ***CREBBP*** ,***CDKN2A/B***,the 6p22 histone gene cluster ***IKZF3*** and ***PAG1***

- **low hypodiploid (31 to 39 chr)** with **TP53 mutations**

- **B-cell lymphoblastic leukemia/lymphoma with** hyperdiploidy

- B-cell lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2)[BCR-ABL1]

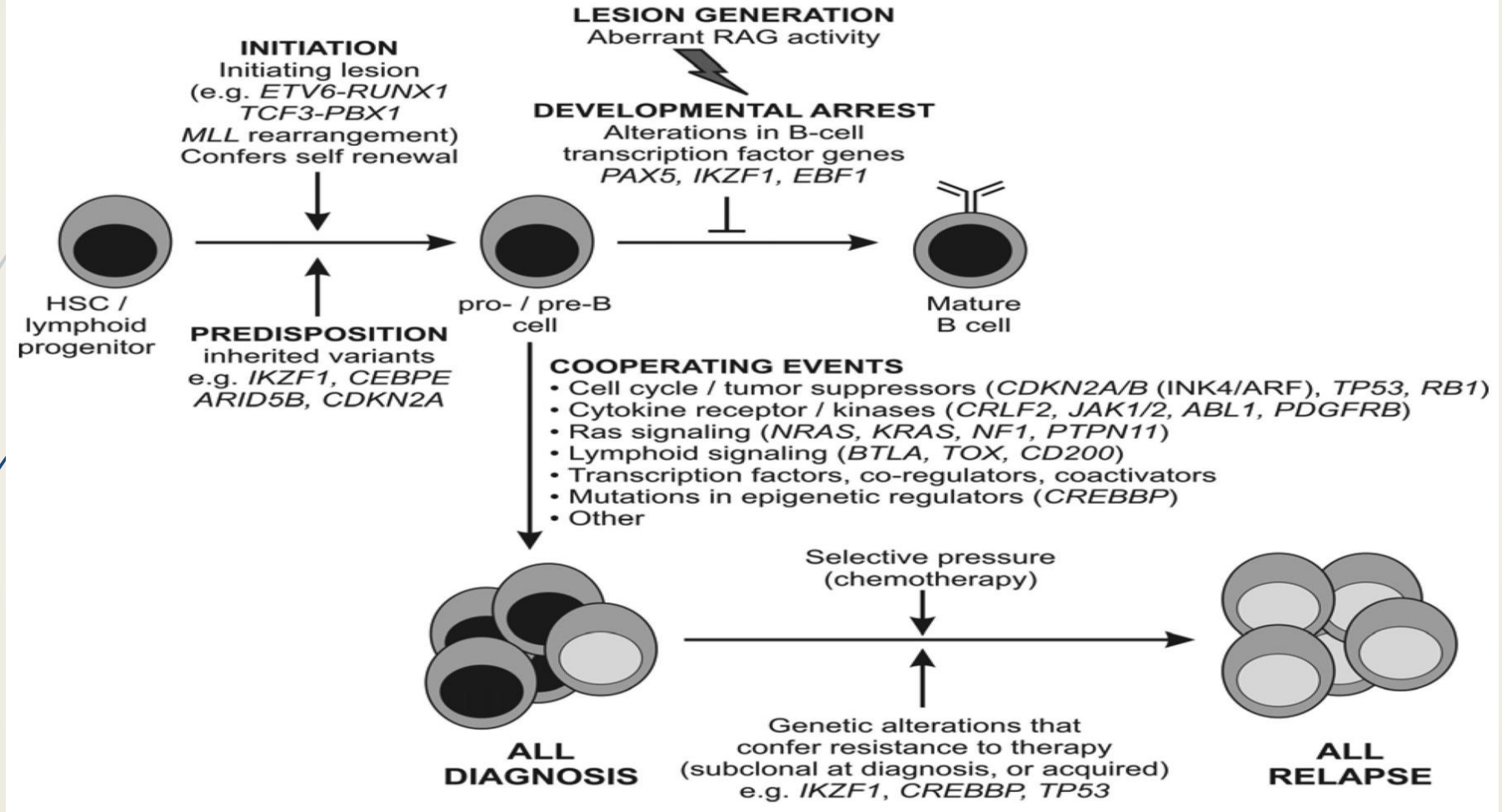
Added entities to B-Cell malignancies WHO 2016 revision

- B-cell lymphoblastic leukemia/lymphoma with **TCF3-HLF t(17;19),**
- B-cell lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32)[**IL3-IGH**] B-cell

provisional entities of B-Cell Lymphoblastic Leukemia

- Two new entities
- **(iAMP21)** Intra chromosomal amplification of chromosome 21
- **BCR-ABL1–like ALL subgroup** B-cell lymphoblastic leukemia/lymphoma with translocations involving tyrosine kinases or cytokine receptors **('BCR-ABL1–like ALL')** .

Proposed schema for the role of genetic alternations in the pathogenesis of BALL.Mullighan and Downing



Common Recurrent Abnormal Fusion Genes in our study

- This study was conducted on **843** pediatric patients **<= 18 years old** with acute B- precursor lymphoblastic leukemia**(BCP_ALL)**
- Referred to **Gholhak clinical laboratory** from (march 2009 to May 2020) from different university hospitals or private clinics in Iran
- To detect the **frequency of common abnormal fusion** genes initiating leukomogenesis in patients with BCP_ALL

Our Study:

- This was a retrospective study on **843** newly diagnosed BCP_ALL
- which was performed on **2 ml bone marrow** aspiration samples collected on EDTA anticoagulant
- Analyzed by **reverse transcription-polymerase chain reaction** (RT-PCR) technique for molecular diagnosis of abnormal fusion genes
- In a period of about **11 years** (march 2009 to May 2020) with
- The age range of (3mth–15 years) and a mean of **7 years**
- Sex(564 males,278 females) **(M: F =2: 1)**

Result :

- Bone marrow aspirate samples of 843 of pediatric BCP_ALL have been analyzed by **reverse transcription-polymerase chain reaction** (RT-PCR) technique

- Abnormal fusion genes which was observed in **191/843 (22%)** of cases and in **652(88%)** of cases was negative for these cytogenetic abnormalities in molecular level .

- From the 191 positive cases,120 had **ETV6-RUNX1 (TEL-AML1**)(14%), 26 had ***BCR–ABL p190*** (3%) and 2 1had **KMT2A-MLL1(*MLL–AF4*)** (2.4%),24 had **TCF3-PBX1 (E2A-PBX1)** (2.5%) fusion genes.

CONCLUSION:

- RT-PCR is a **sensitive technique** in detecting fusion transcripts of oncogenes with significant prognostic roles.
- In our study of 843 pediatric patients with BCP-ALL,
- A lower frequency of **ETV6-RUNX1 (TEL-AML1)**(14%) in comparison with western countries (15-25%)
- A higher frequency of **KMT2A-MLL1(*MLL–AF4*)** (2.4%) which is (1-2%) in western literature was observed

CONCLUSION:

- Comparable with western studies on childhood BCP- ALL, our study showed male predominance, with the male-to-female ratio being **2 : 1**.

- The frequency of aberrations in our population except for **KMT2A-MLL1 and ETV6-RUNX1** is almost similar with the western literature

- **We may conclude that a multicenter data analysis and may**

be a higher population of patients should be investigated to give a better perspective of genetic aberrations in Iranian population

Common recurrent cytogenetic Abnormalities in molecular level

Anita CHOPRA ET.AL 2015/Charles G. Mullighan 2012

Fusion gene	Our study	European Study	Indian study
ETV6-RUNX1 (TEL-AML1)(t(12;21)	14%	15-21	(7.3%)
KMT2A-MLL1(*MLL–AF4* t(4;11)	2.4%	1-2	(1.4%)
TCF3-PBX1 (E2A-PBX1)t(1;19)	2.5%	2-6	(5.9%)
BCR–ABL p190 _x0002_**t(9;22)**	3%	2-4	(11.9%)

Pediatric ALL cooperative trial groups Use Some Combination of Clinical Risk Factors

Current prognostic algorithms rely on prognostic variables such as

- Age and WBC
- MRD response
- Tumor genetics
- To stratify patients into different risk groups that receive more or less intensive chemotherapy and/or alternative therapies, such as allogeneic stem cell transplantation, in first complete remission (CR1).

High-risk features in pediatric acute lymphocytic leukemia (ALL)

- Age **less than 1 year old** or greater than 10 years old
- Initial white blood cell count greater than 50,000/μL
- Central nervous system involvement
- Testicular involvement
- Unfavorable cytogenetics (hypodiploidy, t(9;22), 11q23, iAMP21)
- Suboptimal induction response (induction failure or positive minimum residual disease)

Risk Stratification According to Cytogenetics And Molecular

- **Favorable outcome :**
- Patients harboring t(12;21)ETV6-RUNX1
- High hyperdiploidy (51-65 chromosomes)
- Trisomy 4,7,10
- DNA index of greater than **1.16**,

Risk Stratification According to Cytogenetics And Molecular

- **UN favorable outcome :**
- t(9;22)(q34;q11.2)/BCR-ABL1
- MLL rearrangements,
- Hypodiploidy or fewer than **44 chromosomes** or a **DNA index of less than 0.81**
- Near haploidy (30 chromosomes),
- low hypodiploidy (30-39 chromosomes),
- Intrachromosomal amplification of chromosome 21 (iAMP21),
- t(17;19)(q23;p13) have an inferior outcome when treated with standard therapy.

Risk Stratification According to Cytogenetics And Molecular

- In **near-haploid (24–31 chromosomes) ALL**, **alterations in tyrosine kinase** or **Ras signaling** was seen in 71% of cases and in IKAROS family zinc finger 3 **(IKZF3)** in 13% of cases.

- In contrast, **low-hypodiploid (32–39 chromosomes) ALL**, **alterations in p53** (91%), **IKZF2** (53%) and ***RB1*** (41%) were more common.

- Both **nearhaploid** and low-hypodiploid exhibited activation of **Ras**- and **PI3K PI3K-signaling pathways -signaling pathways**, suggesting that these pathways may be a target for therapy in **aggressive hypodiploid ALL**.

Which markers have the most significant impact for risk stratification

- t(9;22)(q34;q11)/*BCR-ABL1*
- *rearrangements of the MLL gene* t(4;11)(q21;q23)/*MLL-AFF1 (previously known as MLL-AF4)*
- *The prognosis of the other MLL partners may become significant in the future, particularly among infants.*
- Detection of these two abnormalities provides the basic criteria for the classification of **high Risk groups**, which is applied in all American and European protocols

Which markers have the most Significant impact for risk stratification

- Other significant structural abnormalities include t(12;21)(p13;q22)/*ETV6-RUNX1*
- fusion, as well as t(1;19)(q23;p13.3)/*TCF3-PBX1 fusion*.
- *Are not used in risk in* stratification on all protocols.
- The *ETV6-RUNX1 fusion occurs in approximately 25% of*
- younger children with BCP ALL and these
- patients have an extremely good prognosis.
- Among patients with *TCF3 rearrangements, those with TCF3- PBX1 were originally regarded*
- As poor risk on some treatment protocols, but on modern therapy they are classified as
- standard risk.
- In contrast the rare variant, t(17;19)(q22;p13)/*HLF-TCF3 fusion, has a*
- **Dismal outcome** on all therapies

Pitt falls of conventional Risk Stratification

- These genetic abnormalities, however, do not entirely account for the outcome as a fraction of patients with favorable genetic features relapse
- Similarly, **a quarter of pediatric ALL patients** who are **standard risk eventually relapse.**
- This indicates that the above-mentioned clinical and genetic parameters are not ideal for risk stratification and therefore **appropriate therapy**.
- In spite of treatment and risk stratification improvement, 20% of children with ALL ultimately relapse
- Cure rate after relapse reaches only **25% to 40%.** Very high remains
- There is an interest to understand the mechanisms of relapse.

Chromosome 21 Abberations

- Intrachromosomal amplification of chromosome 21 (iAMP21) consists of an abnormal
- Amplified region that in all cases includes the *RUNX1 gene*.
- *This abnormality was* originally described as poor risk factor, although the outcome has since been shown to be protocol dependent
- Other aberrations involving chromosome 21 in ALL are the t(12;21) with good prognosis

Cytogenetic classification

TranslocationsinALL	Prognosis
t(12;21)	Good
t(1;19)	Poor
t(4;11)MLLfusion	Poor
JAK-2Mutation	Poor
t(9;22)BCR-ABL	Very Poor

Genomic technologies have identified

- **Novel copy number alterations (CNAs)**
- And sequence mutations that typically affect genes involved in **lymphoid differentiation, proliferation, cell cycle, and transcription**.
- In contrast to chromosomal abnormalities, which are **commonly initiating events**,
- These CNAs are usually **cooperating aberrations** that correlate with **specific cytogenetic subtypes**

Genomic technologies

- Have identified a plethora of Novel Copy Number alterations (CNAs) and sequence mutations
- That typically affect genes involved in
- Lymphoid differentiation,
- Proliferation,
- Cell cycle,
- Transcription.
- In contrast to chromosomal abnormalities, which are commonly initiating events, these CNAs are usually cooperating aberrations that correlate with specific cytogenetic subtypes

Copy number alternation

- In the last 50 years, we have seen a revolution in the responses of childhood acute lymphoblastic leukemia (ALL) to chemotherapy
- Improving event-free survival from <10% in the 1950s to more than 90% in the last decade.
- These changes have been brought about by an in-depth understanding of chemotherapy and leukemia biology
- As well **As treatment of ALL** patients based on risk stratification

Submicroscopic Genomic Alterations in BCP-ALL

- We now recognize that there are **recurrently occurring DNA sequence mutations**
- As well As **copy number alterations (CNAs)** **affecting key B-cell developmental pathways** in B-lineage ALL (B-ALL Copy number alternations (**CNAs)**
- **cooperating with initial cytogenetic abnormalities** involving
- ***IKZF1–3*** **,*PAX5*** **,*CDKN2A/CDKN2B*, *RB1*, , *ETV6*,** and ***ERG*** genes or locus
- These abnormalities can play a major role in risk stratification in **BCP_ALL**

Candidate gene sequencing Techniques :

- **MLPA**
- **Microarray**
- **Genome wide sequencing (GWAS)**
- **Next Generation sequencing(NGS)** sequencing techniques
- these techniques Has to be incorporated in to the contemporary technologies
- To give us **a better picture of BCP-ALL genomic alternations in pediatric BCP_ALL patients in Iran**

Submicroscopic deletions detection by MLPA

- Successfully applied in simultaneous screening of those genes most frequently deleted in high risk BCP-ALL,
- Including the B-cell differentiation genes:IKZF1, PAX5, EBF1 and BTG1
- Cell cycle control genes: RB1 and CDKN2A/B and those deleted from the PAR1 region:
- MLPA can accurately detect deletions in all these genes, which are present in more than 20% to 30% of cells.

Multiplex ligation-dependent probe amplification (MLPA)

- Is a molecular technique developed by MRC-Holland back in 2002.
- MLPA is a sensitive technique that allows quantification of nucleic acid sequences, quickly and efficiently.
- Detect copy number changes (like deletions or duplications) of a gene
- Identify the methylation status of DNA,
- Detect single nucleotide polymorphisms (SNPs) and point mutations
- Quantify mRNA.
- Therefore, it is used in many research and diagnostic fields, such as Cytogenetics,
- Cancer research,
- Human genetics, among others.

Multiplex ligation-dependent probe

- Genomic DNA is extracted from the diagnostic material
- The **SALSA MLPA** (MRC-Holland, the Netherlands) will be used to detect CNA in B-ALL following the manufacturers recommendations.
- Data is analyzed using the Coffalyzer software(MRC-Holland, Amsterdam, The Netherlands).
- Patients are divided into **good**- and **poor-risk genetic abnormalities** to stratify them according to the **integrated genetic profile as described by Moorman *et al*.**
- Cytogenetic abnormalities took precedence over CNA abnormalities.

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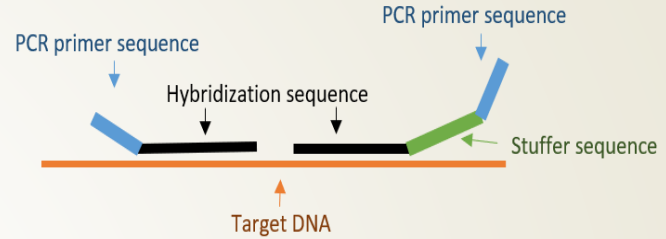
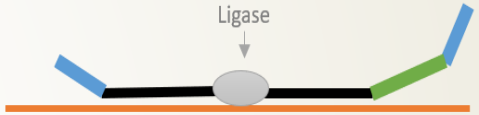
How does MLPA works?

- MLPA consists of the following steps
- Denaturation
- Hybridization
- Ligation
- Amplification (by PCR)
- Fragment Separation and Data Analysis

1 – Denaturation; 2 – Hybridization

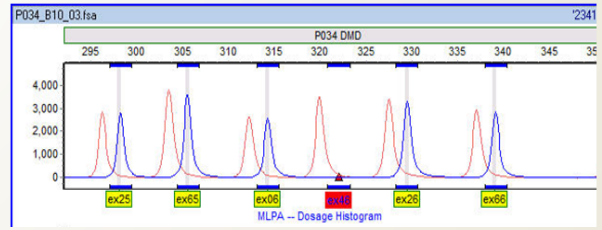


3 – Ligation



4 – Amplification

5 – Fragment separation and Data analysis



Genomic technologies have identified

- Mullighan *et al.* studied a large cohort of B-ALL patients and found recurrently occurring CNA in ***IKZF1***, ***PAX5***, and other genes.
- Importantly, they stated that **ALL** harboring ***IKZF1*** mutations were associated with **persistent MRD** values and **poor prognosis**.
- ***BCR-ABL1*-like ALL** was first described by Den Boer *et al* as well as by Mullighan *et al*.
- These are a subset of **ALL** with a unique gene expression profile that resembles ***BCR-ABL1* rearranged ALL**.
- Importantly, they are associated with a **high-risk clinical features** and are more frequently **MRD positive**.

BCR-ABL1-like ALL

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- *BCR-ABL1*-like ALL was first described by Den Boer *et al* as well as by Mullighan *et al*.
- These are a subset of ALL with a unique gene expression profile that resembles *BCR-ABL1* rearranged ALL.
- Importantly, they are associated with a high-risk clinical features and are more frequently MRD positive.
- 10-15% ALL in children

Ph-like ALL

- This has significant therapeutic implications as it suggests that **Ph-like ALL**, which tends to carry a **worse prognosis**, **may respond to kinase inhibitors.**
- In fact, Roberts et al.14 showed that cell lines and human leukemic cells expressing **ABL1**, **ABL2**, **CSF1R** and **PDGFRB** were sensitive in vitro and in vivo human xenograft models to **second-generation TKIs (**for example, **dasatinib**.);
- Those with **EPOR** and **JAK2** rearrangements were sensitive to **JAK kinase inhibitors** (for example,(**ruxolitinib)**
- Those with **ETV6-NTRK3** fusion were sensitive to **ALK inhibitors crizotinib**

B-others

- Improvement in the genetic lesion detection has led to the definition of new ALL
- subtype characterized by new recurrent aberrations,
- such as patients with iAmp21, *ERG* alteration
- or *CRLF2 alterations*
- These new recurrent aberrations reduce the group of genetically undefined patients, called B-others
- few year ago counted for 22% of ALL
- And now are restricted to 7%
- Further dissection of B-others group are expected from the applications of new technologies such as next generation high-throughput sequencing.

Chromosome 21 anomalies

- play an important role in tumor development as acquired somatic mutations.
- 23 % of all chromosomal abnormalities in ALL involve:
- Trisomies or tetrasomies of chromosome 21.
- 90% percent of childhood ALL with chromosome numbers >50 show trisomy or tetrasomy 21
- But also 30% of B-cell ALLs with a chromosome number of 47–50 present with > 2 chromosome 21 copies

CNAs of Genes Being Addressed by MLPA

- BTG anti-proliferation factor 1 (BTG1)
- Early B-cell factor 1 (EBF1)
- Cyclin dependent kinase inhibitor 2A/2B (CDKN2A/2B)
- Cytokine receptor like factor 2 (CRLF2),
- Interleukin 3 receptor subunit a (IL3RA)
- Colony-stimulating factor 2 receptor a subunit (CSF2RA)
- Short stature homeobox (SHOX) genes.
- Intra-chromosomal amplification of chromosome 21iAMP21
- ERG intragenic deletion (ERG) with aberrant expression of cd2

CNAs Being Addressed by MLPA

- **IKAROS family zinc finger 1 (IKZF1)**
- **purinergic receptor P2Y8 (P2RY8),**
- **Zinc finger protein, Y-linked (ZFY)**
- **Janus kinase 2 (JAK2),**
- **Paired box 5 (PAX5)**
- **ETS variant 6 (ETV6)**
- **RB transcriptional corepressor 1 (RB1),**

GENE EXPRESSION PROFILING

- Today approaches in biomedical research often employ high throughput technologies in
- Comprehensive studies named genomics, proteomics, metabolomics (etc.)
- The "Omic" studies disclose molecular correlation Networks that are the basis of normal and pathogenic cellular processes

The power of genomics applying microarray technology :

- Tens of thousands of genes are analyzed simultaneously and without predetermined bias
- Novel genes Involved in disease processes can be discovered
- Develop not only new diagnostic markers but also prognostic and disease progression Markers, ultimately leading to the prospect of patient-tailored therapy

MICROARRAY TECHNOLOGY

- Microarrays consist of numerous regularly spaced DNA probes which are immobilized on a solid surface.
- The pool of transcripts in a given patient sample is labelled with a fluorescent dye and hybridized to the microarray.
- The fluorescent signal bound to the probe serves as an indicator of the expression of the corresponding transcript

MICROARRAY TECHNOLOGY

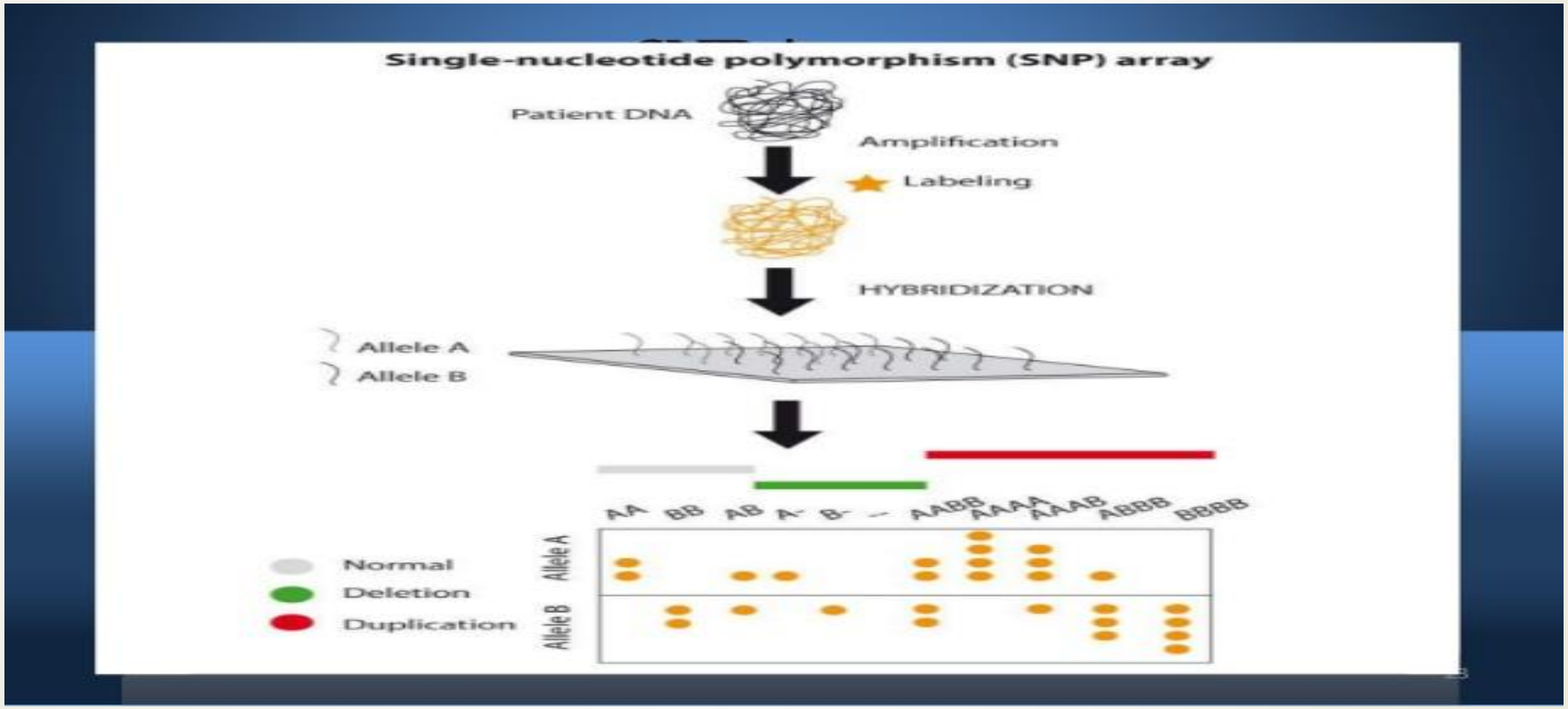
- Most laboratories use DNA-oligonucleotide microarrays of 25–60 nucleotide length as this minimizes the risk of cross-hybridization and guarantees a high level of specificity.

- Currently available commercial arrays have all reached whole transcriptome coverage

Application of microarray technology

- Initially has aimed at evaluation **of expression of coding sequences of genes** by assessment of the **amount of RNA transcription (gene expression profiling, GEP).**
- Subsequently their use has been extended to investigation of non coding sequences e.g. microRNAs expression **(miRNAs profiling).**
- Another widespread application was the quantification of gene dosage on the genomic DNA level
- Allowing to explore single nucleotide polymorphisms (SNPs)
- Copy number alterations
- (copy number variations, loss of heterozygosity (LOH), and copy number neutral LOH due to uniparental disomy UPD) in parallel
- Currently available arrays incorporate up to **~2.7 million SNP** or **copy number probes**
- However, **SNP analysis is not capable of directly identifying reciprocal translocations** which commonly occur in AL **(Acute Leukemia).**

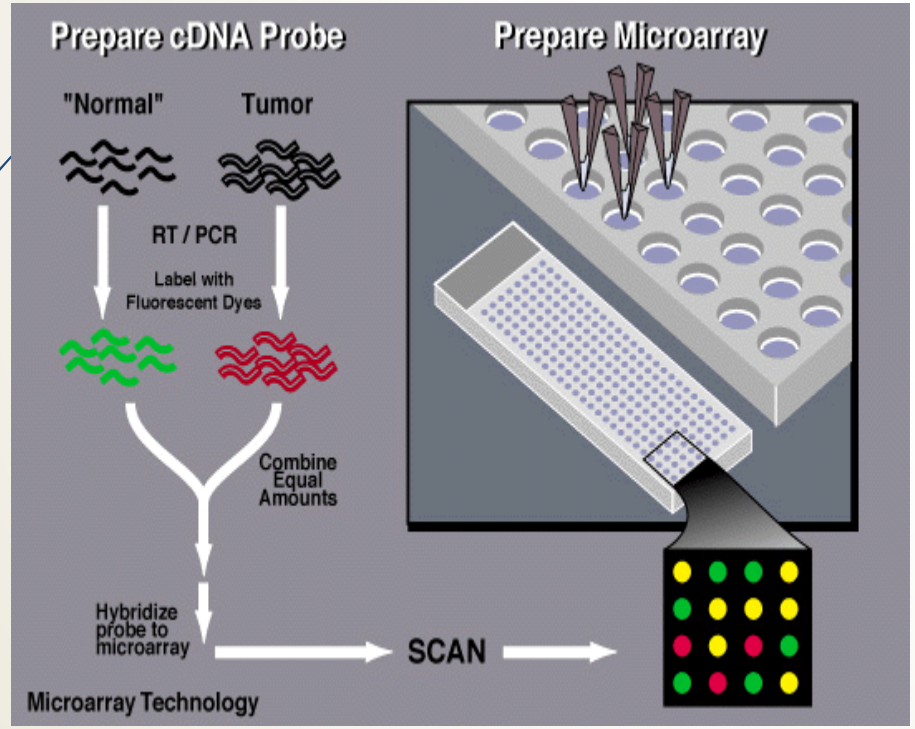
SNP array is a type of DNA Microarray which is used to detect polymorphism within a population

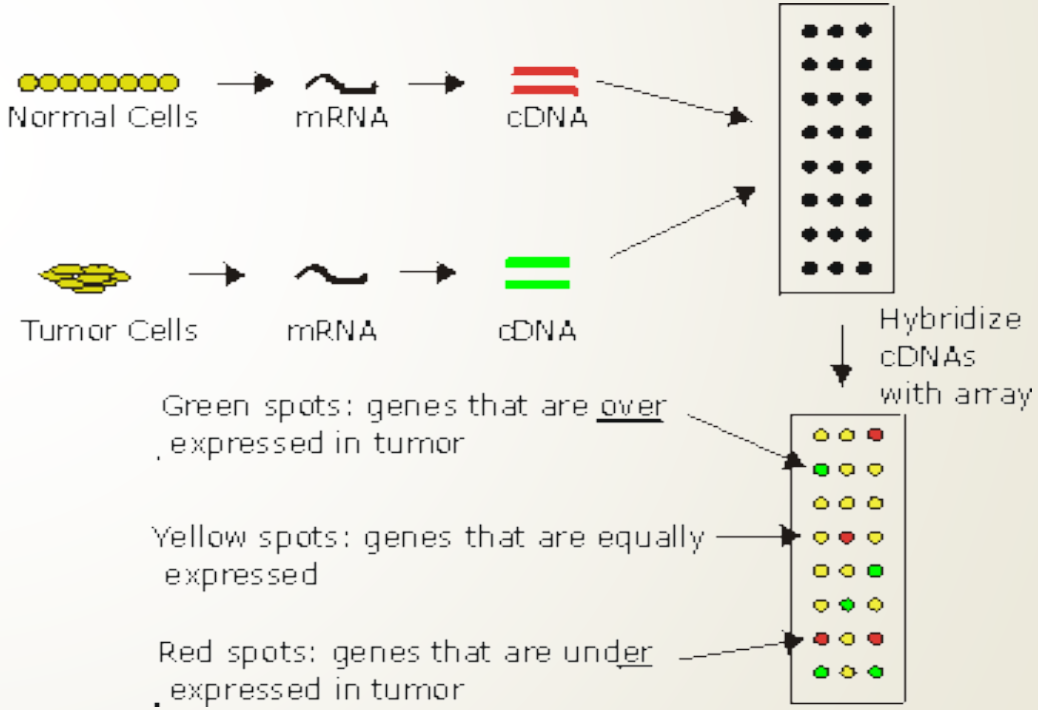


DNA arrays provide a new and potentially very powerful set of tools for the analysis of DNA and gene expression.

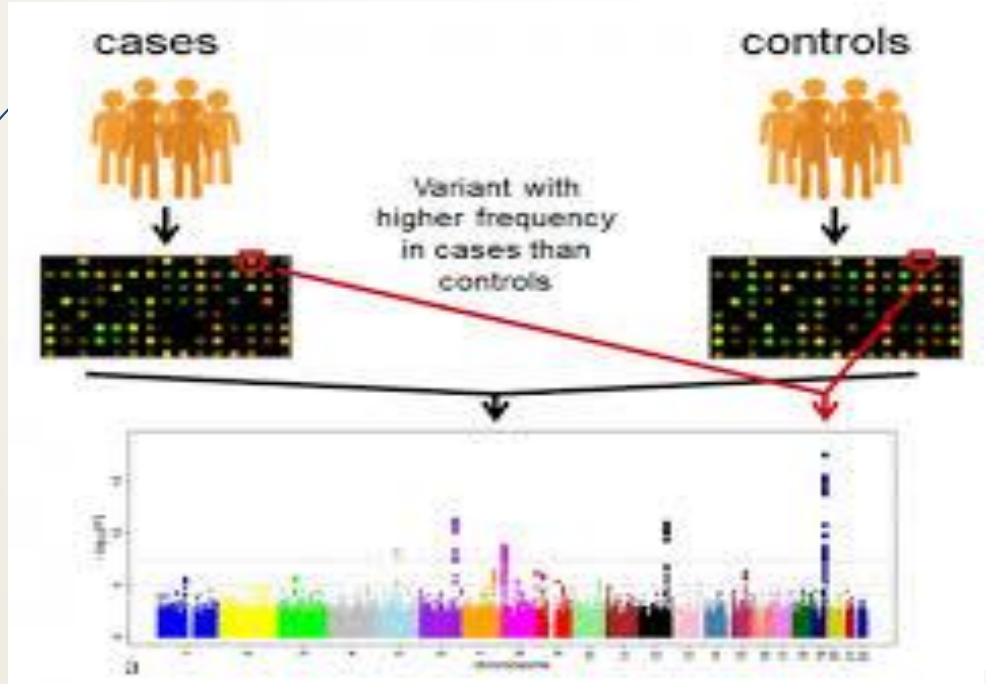
A DNA array consists of thousands of different DNA sequences placed on a substrate (such as a glass microscope slide).

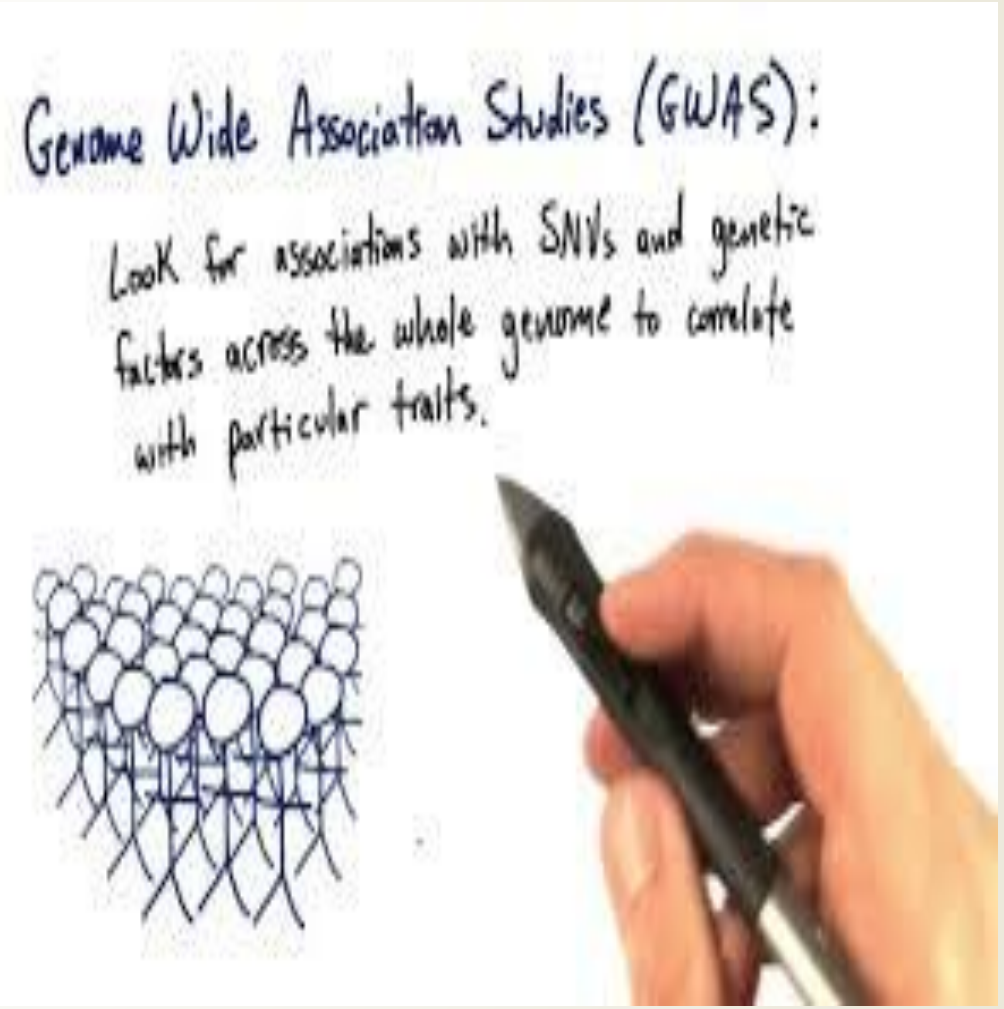
Example: measure the changes in gene expression between normal cells and tumor cells.
This application is currently being used to identify **cancer related genes**, and has the potential for being useful in the diagnosis and staging of tumors.





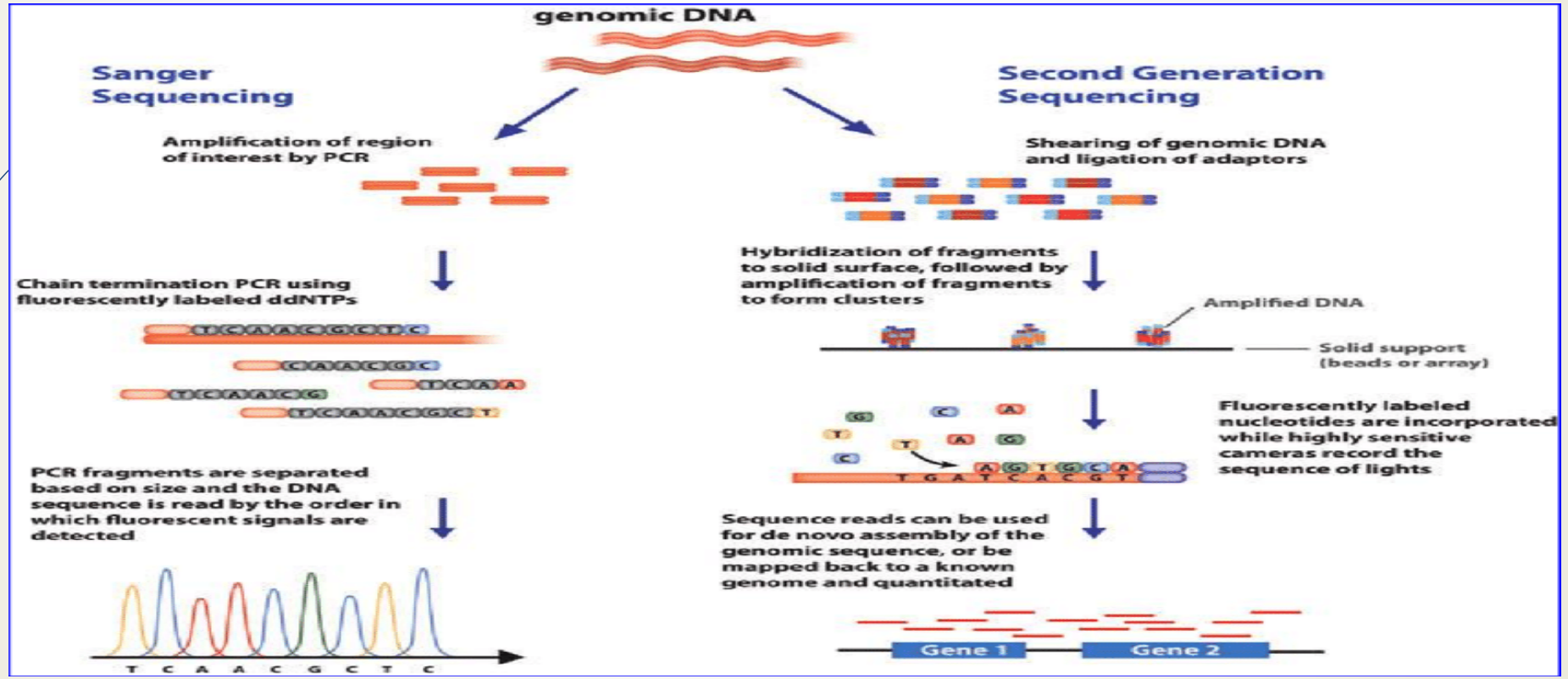
Genome wide association studies (GWAS) are hypothesis-free methods for identifing associations between genetic regions (loci) and traits (including diseases).





Comparison between Sanger sequencing and next-generation sequencing (NGS) technologies. Sanger sequencing is limited to determining the order of one fragment of DNA per reaction, up to a maximum length of *700 bases.

NGS platforms can sequence millions of DNA fragments in parallel in one reaction, yielding enormous amounts of data.



Good risk genetic abnormalities

- **Good risk genetic abnormalities**

- ETV6-RUNX1 t (12;21)(p13:q22)
- High Hyperdiploidy (51-65 chromosomes)

- **Good Risk Copy Number Alternation profiles**
- No deletion of IKZF1, CDKN2A/B, PAX5, EBF1, ETV6, BTG1, RB1, and PAR1 (CSF2RA/IL3RA/CRLF2)
- Isolaed deletion of IKZF1,PAX5,BTG1
- ETV6 deletion with a single additional duplication of BTG1,PAX5<CDKN2A/B

Intermediate Risk

- Although it means that other CNAs (eg, ERG deletions) and sequence variants (eg, RAS, JAK2, and TP53 mutations26) are not represented,

- it is known that many of these alterations correlate closely with either chromosomal abnormalities or CNAs already represented in the classification.

- For example, RAS mutations with near haploidy/high hyperdiploidy
- TP53 mutations with low hypodiploidy
- JAK2 mutations with P2RY8-CRLF2 (PAR1 deletions).

IKZF1 gene Alternations

- IKZF1 encodes the lymphoid transcription factor IKAROS, which is a key regulator in early lymphocyte development.
- Alterations mainly comprise deletions and only rarely sequence alterations
- Irrespective of the type of IKZF1 alteration, they prevail in poor responding cases in major treatment protocols.
- They are a hallmark of high-risk BCP ALL, especially those, which carry a BCR-ABL1 and other cytokine- and kinase-activating fusions, including IGH-CRLF2 and P2RY8-CRLF2
- The common characteristic of such cases is a gene expression signature that resembles that of genuine BCR-ABL1-positive cases and which are, therefore, also referred to as either 'BCR-ABL1-like' or 'Ph-like'.
- IKZF1 alterations still confer a dismal prognosis in such cases, even when treated on high risk or kinase inhibitor-containing protocols

P2RY8-CRLF2 fusions

- P2RY8-CRLF2 fusions often carry additional alterations in JAK/STAT pathway genes and they may cooperatively activate downstream pathways.
- They are associated with a significantly increased relapse risk in AIEOP/BFM protocols, which is independent of the size of the P2RY8-CRLF2-positive clone.
- Respective cases are primarily classified as non-high risk by clinical and molecular response criteria and relapses occur predominantly late.

CRLF2 Alternations

- over expression of CRLF2, which has been defined as a novel, significant abnormality in BCP-ALL.
- CRLF2 alterations, including activating mutations of the CRLF2 receptor itself, are associated with activating JAK mutations resulting in constitutive activation of the JAK-STAT signaling pathway (Chapiro, et al 2009, Hertzberg, et al 2009, Russell, et al 2009a, Yoda, et al 2009).

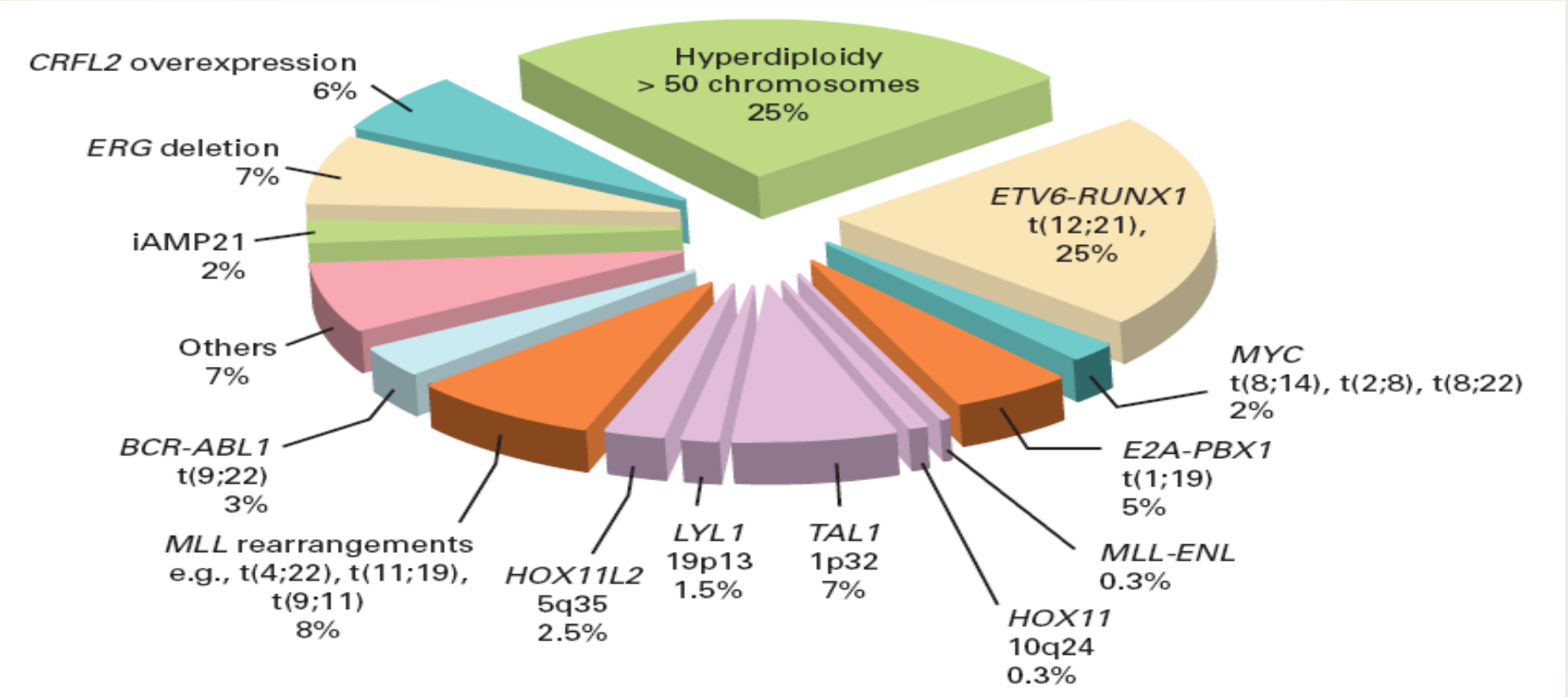
- Activation of this pathway has been associated with a worse prognosis in adults and children (Cario, et al 2010, Harvey, et al 2010) and has been highlighted as an important consideration for targeted therapy.
- Following further validation, the detection of CRLF2 alterations may become a necessary diagnostic test.

iAMP21Prognosis

- Patients with an iAMP21 treated under standard risk
- Regimens had an elevated relapse rate and a dismal prognosis compared to other BCP-ALL, hence risk directed treatment intensification has been recommended
- *(PDF) Evaluation of multiplex ligation dependent probe amplification (MLPA) for identification of acute lymphoblastic leukemia with an intrachromosomal amplification of chromosome 21 (iAMP21) in a Brazilian population*. Available from: https://www.researchgate.net/publication/278044567_Evaluation_of_multiplex_ligation_dependent_probe_amplification_MLPA_for
- _identification_of_acute_lymphoblastic_leukemia_with_an_intrachromosomal_amplification_of_chromosome_21_iAMP21_in_a_Brazilian_pop [accessed Aug 24 2018].

Figure 1. Estimated frequency of genetic abnormalities in ALL. Violet area are referred to T ALL abnormalities,

other colours are referred to B ALL abnormalities (From Pui et al., 2011)55.



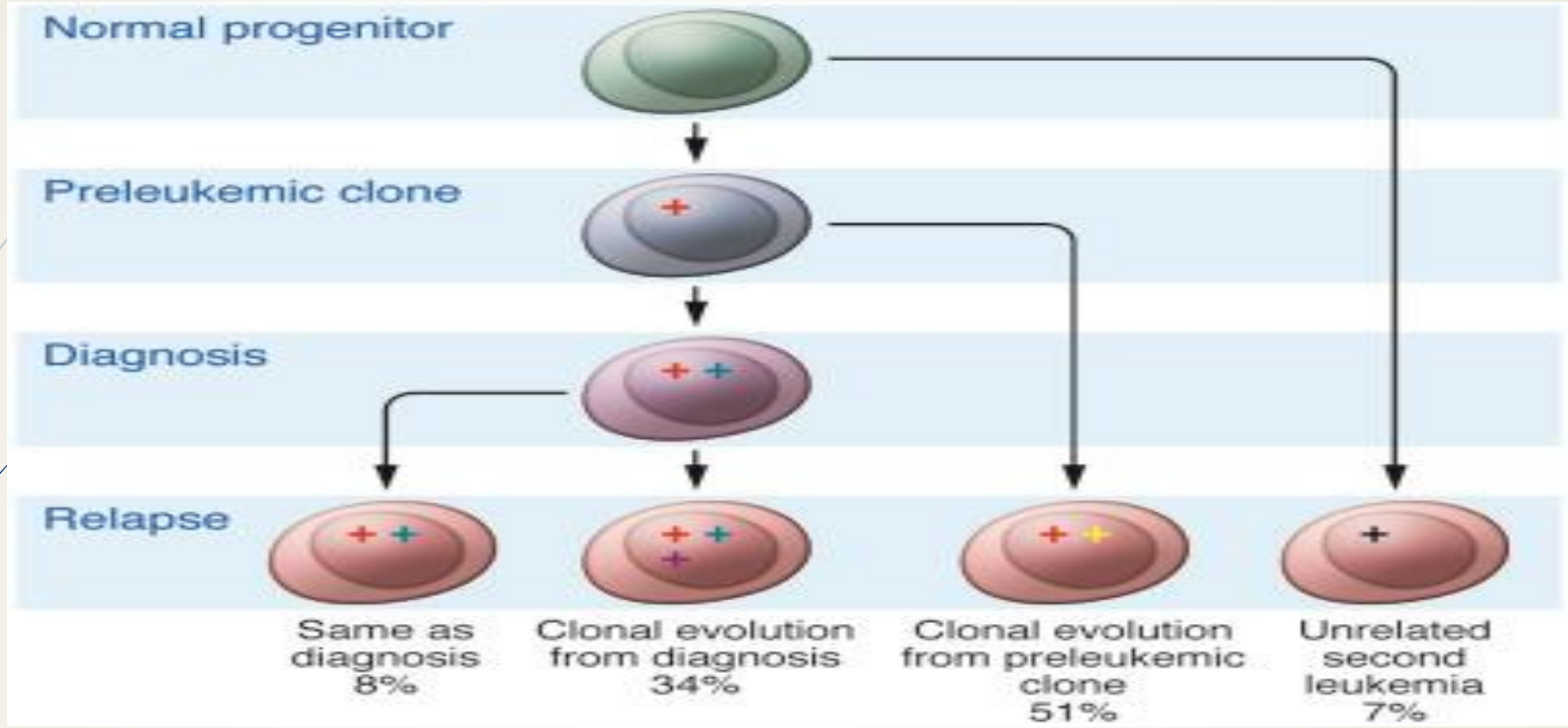
Improvement of survival in childhood ALL

- Survival rates in modern protocols are approaching 90% and thus any change to the treatment approach must be carried out with precaution.
- Nevertheless, a reduction of treatment intensity would reduce the risk of side effects and improve quality of life for the patients.
- Further improvement of survival and quality of life in childhood ALL will likely be obtained through the development of targeted compounds combined with a more personalized diagnostic approach, where treatment decisions are based on the presence **of targetable somatic mutations** and **therapy-relevant constitutional genetic variants** in each child rather than on risk-group stratification
- Acquiring more advanced technologies is for sure a necessity in our country

Treatment Resistence

- Are clonally related, and that the relapse clones are often present as minor populations at Diagnosis,
- which suggests that they are selected during treatment.
- Indeed, many of the genetic Alterations that emerge in the dominant clone at relapse involve genes that have been

- Implicated in treatment resistance (e.g. *CDKN2A/B or IKZF1) and gene expression studies*
- Have identified a proliferative gene signature that emerges at relapse with consistent upregulation of genes, such as survivin, that could provide useful targets for novel therapeutic intervention.



Leukemic clones at relapse are frequently related to leukemic clones present at diagnosis.
In more than half of cases, the relapse clone arises from a clone present prior to diagnosis, retaining some but not all of the lesions found at diagnosis and containing some additional mutations.
The relapse clone is often present as a rare subclone within the diagnostic sample.
Alternatively, cases of relapse can stem from the diagnosis clone, acquiring additional mutations.
Less commonly, the relapse clone may be identical to the diagnosis clone or appear to be an unrelated second leukemia.

conclusion

- integrated cytogenetic and CNA data into a single genetic classification t can be used to refine patient treatment according to more detailed genetic description of their leukemia.
- GENGR patients have an excellent outcome with modern therapy, and future treatment approaches should focus on achieving the same results with reduced therapy.
- Although, GEN-PR patients have an inferior outcome compared with GEN-GR patients, their survival still exceeds 80%.

- As many of these patients are already receiving intensive treatment,
- further genetic research is required to identify patients curable with current drugs and those requiring alternative therapies.