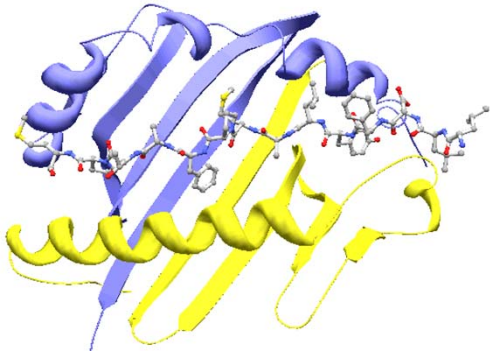
10th INTERNATIONAL SUMMER SCHOOL ON IMMUNOGENETICS
15-18 SEPTEMBER 2013, STINTINO/SARDINIA, ITALY

SESSION 2: NK CELLS, KIRs, MINOR ANTIGENS, AND SNP's

Monday, September 16, 2013

SNP's in Transplantation



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Human Genetic Variation

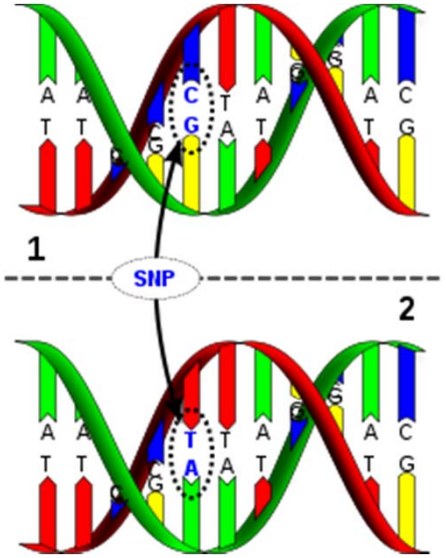
What makes us unique

In 3 billion bp of DNA in the human genome, inter-individual variability is 0.5%

Human Genetic Variation

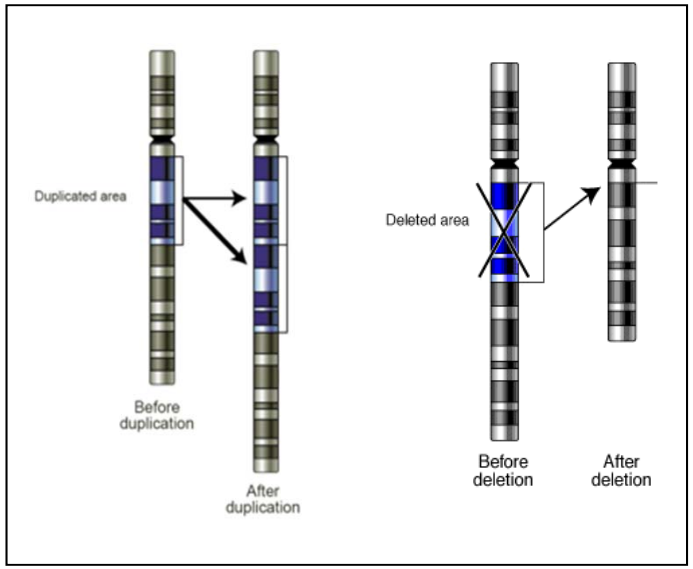
SNP and CNV

Single Nucleotide Polymorphisms (SNP)



- 0.1% of inter-individual variability
- 30 x 10^6 SNP are known
- 1% are functional ($3x10^5$)

Copy Number Variations (CNV)



- 0.4% of inter-individual variability
- Insertion, Deletion, Inversion, Duplication

Detection: PCR-SSP; PCR-SSO; SNP Arrays; NGS

Polymorphic Gene Systems

Biological and Clinical Role

Genetic Polymorphism	Biological Relevance	Clinical Relevance
Classical HLA class I+II	Antigen Presentation Alloreactivity Innate Immunity Evolution	Transplantation Autoimmunity Infectious Disease Pregnancy
Non-Classical HLA (HLA-G)	Immunological Tolerance	Pregnancy Transplantation
Minor Histocompatibility Antigens	Alloreactivity	Transplantation Pregnancy
Natural Killer Receptors (KIR)	Innate Immunity	Infectious Disease Transplantation
Cytokine Genes and Receptors	Immune Response	Transplantation Autoimmunity Infectious Disease
Blood Groups	Natural Immunity	Transfusion Medicine
Adhesion Molecules (CTLA-4)	Immune Response	Transplantation Autoimmunity Infectious Disease
Others (PTX3, PTTN2, etc.)	Cell activation Signal Transduction	Infectious Disease Autoimmunity

Polymorphic Gene Systems

Role in Transplantation

Association with Clinical Outcome

- **HLA-A, B, C, DRB1**
- HLA-DRB3/4/5; DQ; DP
- KIR
- Minor Histocompatibility Antigens
- Non-Classical HLA (G, E)
- Cytokines and Cytokine Receptors

Statistical Associations targeting indivdual polymorphisms or GWAS

Diagnostic Use for host-donor Chimerism

- **Microsattelites (STR)**
- SNP (qPCR)
- Indels (qPCR)

SNP's and Transplantation Outcome

An attractive Hunting Ground

How different in the MHC region are 2 HLA-identical unrelated individuals ?

High throughput sequencing of 3.5×10^6 bp MHC of a patient and his HLA 10/10 compatible unrelated donor

3025 different positions (pat vs. don)
1492 in intergenic regions
1173 in introns
360 in exons (59 genes)

Pröll et al. DNA Res. 2011; 18: 201

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Why Most Published Research Findings Are False

John P. A. Ioannidis

Summary

There is increasing concern that most current published research findings are false. The probability that a research claim is true may depend on study power and bias, the number of other studies on the same question, and, importantly, the ratio of true to no relationships among the relationships probed in each scientific field. In this framework, a research finding is less likely to be true when the studies conducted in a field are smaller; when effect sizes are smaller; when there is a greater number and lesser preselection of tested relationships; where there is greater flexibility in designs, definitions, outcomes, and analytical modes; when there is greater financial and other interest and prejudice; and when more teams are involved in a scientific field in chase of statistical significance. Simulations show that for most study designs and settings, it is more likely for a research claim to be false than true. Moreover, for many current scientific fields, claimed research findings may often be simply accurate measures of the prevailing bias. In this essay, I discuss the implications of these problems for the conduct and interpretation of research.

A research finding is less likely to be true with:

-smaller effect size
-greater nb of tested relationships
-greater flexibility in design, definitions,
outcomes, analytical modes
-more teams involved in the field
-financial interest

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Sign of the Zodiac as a Predictor of Survival for Recipients of an Allogeneic Stem Cell Transplant for Chronic Myeloid Leukaemia (CML): An Artificial Association

R.M. Szydlo, I. Gabriel, E. Olavarria, and J. Apperley

Transplantation Proceedings, 42, 3312–3315 (2010)

Table 2. Probabilities of Survival at 5 Years After SCT for Zodiac Star Signs

Parameter	*n*	Probability of Survival	*P*
Star sign (calendar dates D/M)			.65
Aries (21/3–19/4)	44	62.9	
Taurus (20/4–20/5)	43	58.1	
Gemini (21/5–20/6)	54	56.8	
Cancer (23/7–22/8)	63	48.8	
Leo (23/7–22/8)	59	57.3	
Virgo (23/8–22/9)	54	46.3	
Libra (23/9–22/10)	54	51.0	
Scorpio (23/10–21/11)	56	59.6	
Sagittarius (22/11–21/12)	54	48.1	
Capricorn (22/12–19/1)	61	56.3	
Aquarius (20/1–18/2)	35	44.5	
Pisces (19/2–20/3)	49	49.7	
Star sign groups			.007
Group A (Aries, Taurus, Gemini, Leo, Scorpio, Capricorn)	317	58.3	
Group B (Cancer, Virgo, Libra, Sagittarius, Aquarius, Pisces)	309	48.1	

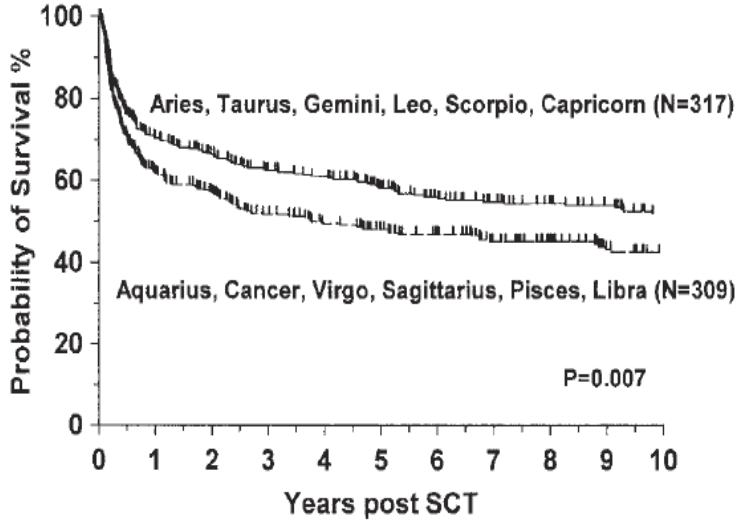

Fig 2. Probabilities of survival after SCT for patients born under the different star sign groupings.

'Statistical analyses should thus be carried out on *a priori* hypotheses and not to find a meaningful or significant result'

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SNP Structure and Function

Association Studies

- Homogeneity of Study Group
- Statistical Power (Under- and Overpower!)
- Statistical Methods
- May lead to refined donor selection

Individual polymorphisms or GWAS Fishing Experiments?

Biological or Clinical Targets

- Functional Rationale
- Can be verified ex-vivo and/or clinically
- May lead to targeted therapies

NOD2 polymorphism

Innate immunity to commensal/pathogenic bacteria of the gastrointestinal microflora
NOD2 = intracellular receptor sensing muramyl-dipeptide from Gram+/Gram- bacteria

Table 1: Summary of published studies on NOD2 SNPs and outcome following SCT.

	Type of SCT	Association	Comment	Refereces
Holler 2004	Related	GvHD, TRM	Single centre	[17]
Holler 2006	Related	GvHD, TRM, OS	Multicentre; Impact of decontamination	[18]
Granell 2006	Related	TRM, pulmonary compl.	CD34 selected grafts	[19]
Sairafi 2008	Related	No association	Low frequency of NOD2 variants	[22]
Hanssen 2008	Related	Weak with GvHD		[20]
Van Velden 2009	Related	Strong with GvHD	Partially T depleted Grafts	[21]
Hildebrandt 2009	Related and Unrelated	Bronchiolitis obliterans		[28]
Mayor 2009	Unrelated	Strong with relapse, not with GvHD	Majority received T-cell depletion With MabCampath	[25]
Holler 2009	Unrelated	Only SNP13 with TRM		[24]
Ngyen 2010	Unrelated	No		[23]

Holler et al. Int. J. Inflamm. 2010

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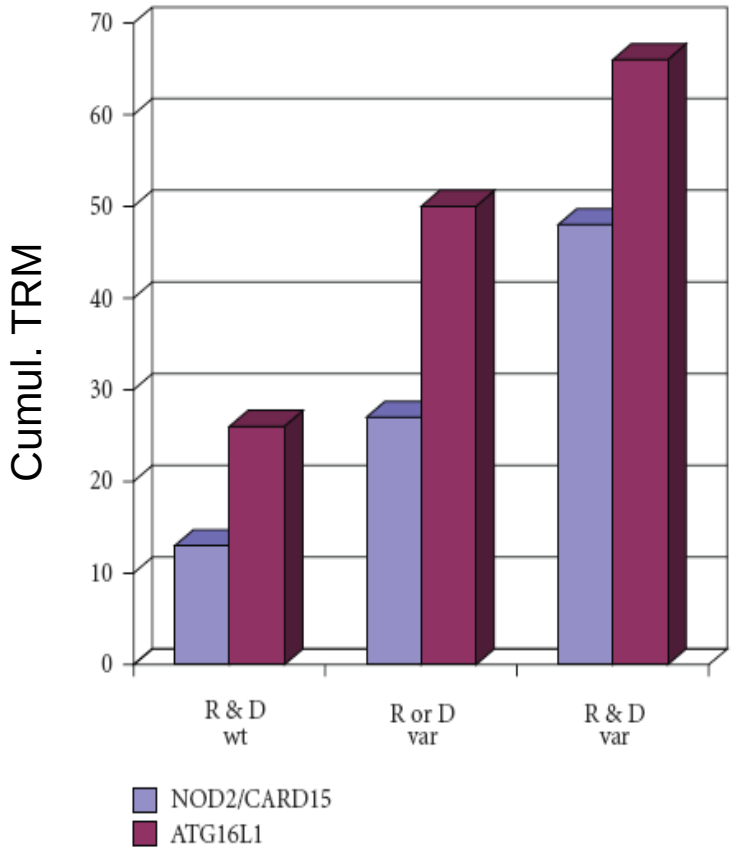
NOD2 polymorphism and TRM



Activation of innate immunity impacts on the adaptative alloreactive immune response: modulation of epithelial inflammation in the gut or bronchi

Holler et al. Int. J. Inflamm. 2010

NOD2 variants (n=358) p=0.003
ATG16L1 variant (n=127) p=0.03
ATG16L1 = autophagy-related gene 1

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SNP's in Transplantation: Chimerism Diagnostics

Chimerism: The She-Goat

- Goat
- Lion
- Snake

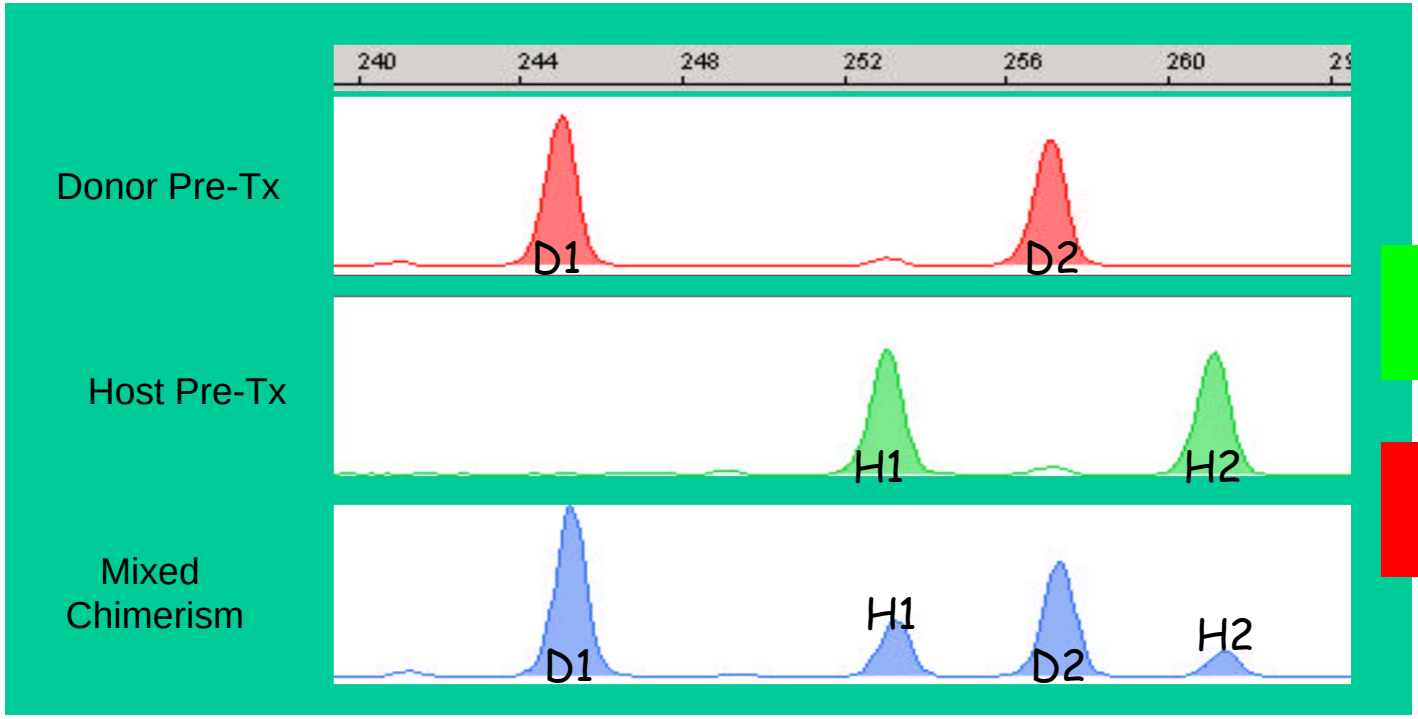
350 B.C. – Musée du Louvre

Co-Existence of cells from two organisms in a single individual

Chimerism

Short Tandem Repeat (STR)

- Multiplex Microsatellite Amplification
- Capillary Gel Electrophoresis
- Relative Quantification

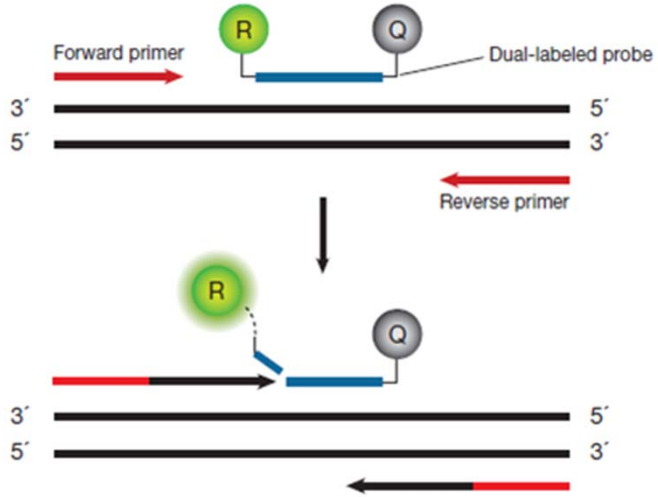


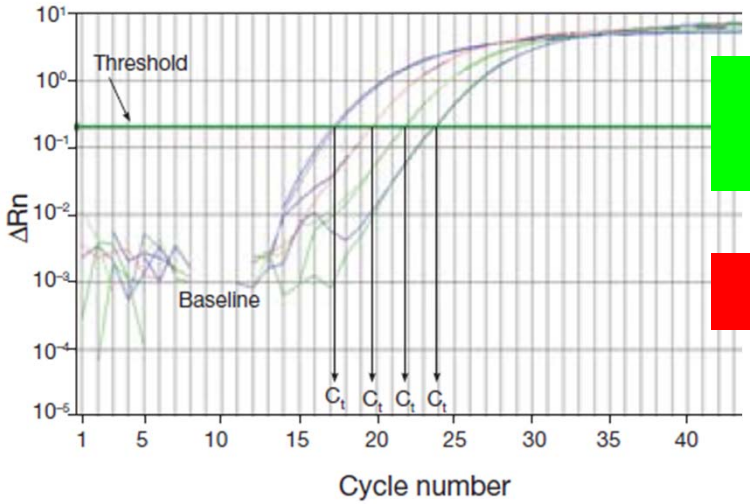
- ng Amounts of DNA
- Fast and Reliable

- 5% Sensitivity
- Primer Competition

Chimerism
Quantitative PCR

- Real-Time PCR
- 34 indel polymorphisms
- Automated Analysis





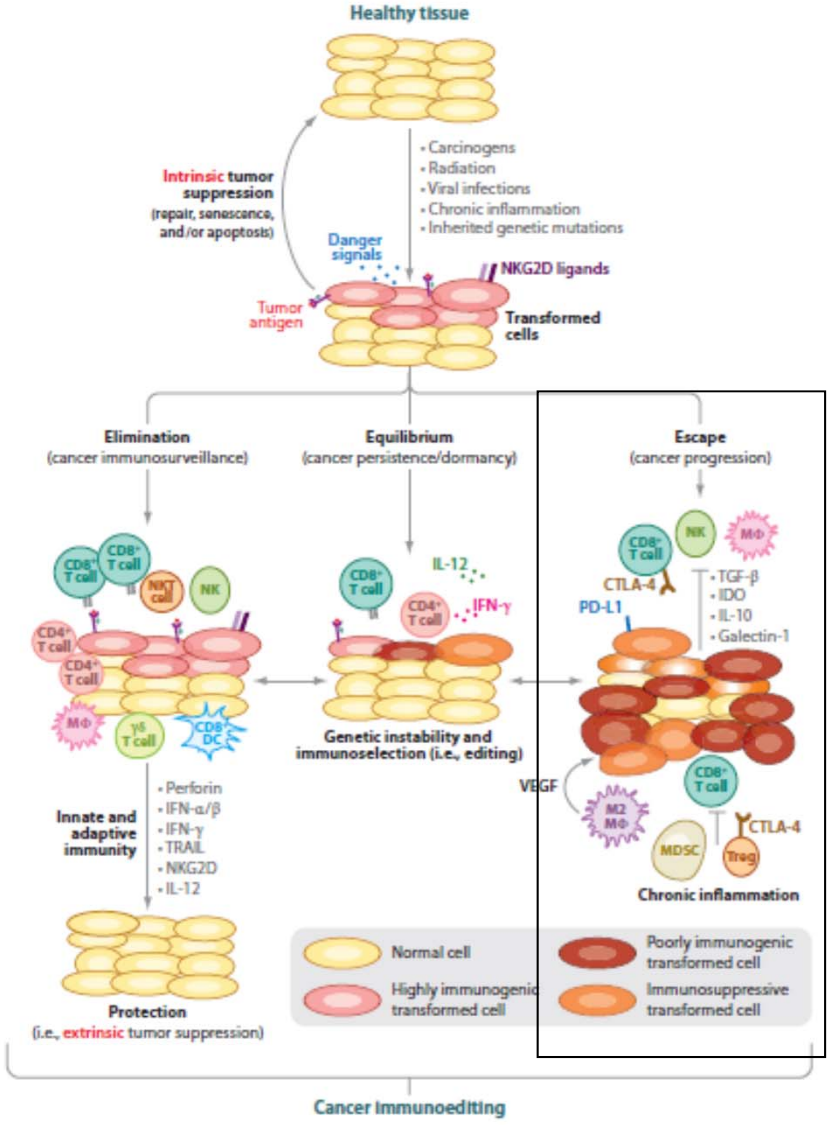
- Fast Interpretation
- Sensitivity <0.1%

- 50ng input DNA

Ct value is proportional to the amount of template

Chimerism for Relapse Detection

Tumor Immunoediting



Downregulation in clonal evolution:

- Immune-regulatory Genes
- Tumor Antigens

Chimerism and Leukemia Cytogenetics

LMA

Alterazione cromosomica	-5	-7	t 15-17	Inv 16	t 8-21
Frequenza (%)	14	14	7	5	3
Marcatore ipoteticamente coinvolto	CA010 CA015 CA025	CA008	CA009 CA016	CA026	CA001

LMC

Alterazione cromosomica	t 9-22
Frequenza (%)	24%
Marcatore ipoteticamente coinvolto	CA030

14/34 indel markers map to cytogenetically relevant chromosomes!

LLA

Alterazione cromosomica	t 9-22	t 1-14	Del 9	Del 17
Frequenza (%)	24	20	11	8
Marcatore ipoteticamente coinvolto	CA030	Ca002 CA006 CA007 CA020 CA034	CA030	CA009 CA016

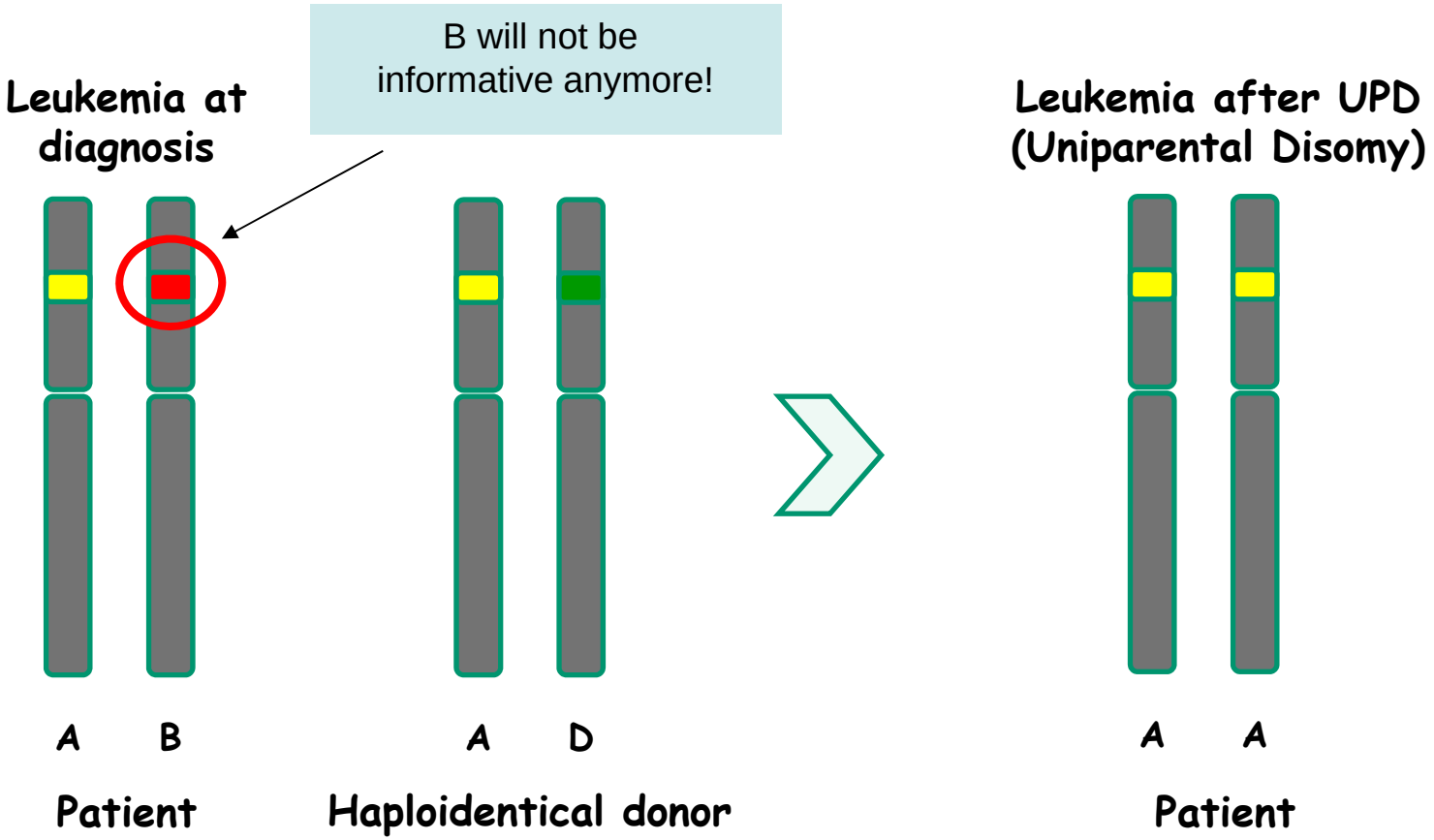
Gaidulis L, Abstract 26-OR 36th ASHI Meeting 2010

ALL relapse post-HSCT undetected by CA029 (chr.2)

Relpase after HLA-Mismatched HSCT

Loss of patient-specific HLA Markers

- 40% of relapses after haploidentical HSCT (Vago 2009)
- Observed also after unrelated HSCT (Toffalori 2012)

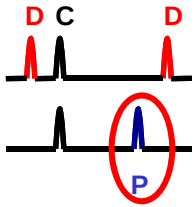


Diagnosis of HLA Loss Relapse

"Split" Chimerism Outside and Inside HLA

Outside HLA: Positive

STR

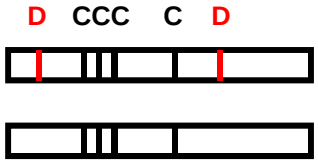


>2bp Duplications

Sensitivity 5%

Inside HLA: Negative

SSO



HLA-A,B,C,DR

Sensitivity 1%

- Diagnosis based on Positive Outside HLA vs Negative Inside HLA
- Minimum Blast percentage 5-10%
- Analysis of sorted blasts preferable
- **Detection in the presence of morphologically overt disease**

Take Home Messages

- Human Genome Variability
  - SNPs, Indels, CNV
  - Any expressed variability can have functional implications
- Association between SNP's and Tx Outcome
  - Caution in Study Design and Statistical methods
  - Structure-Function Correlation can lead to targeted therapies
- Mismatched SNP's as Chimerism Targets
  - Novel qPCR or digital PCR approaches
  - Be aware of Leukemia as a "moving target": SNP's in Leukemia-relevant genes may change during the course of the disease