Le neoplasie del bambino: geni, prevenzione e terapia

Napule è...

PEDIATRIA PREVENTIVA E SOCIALE

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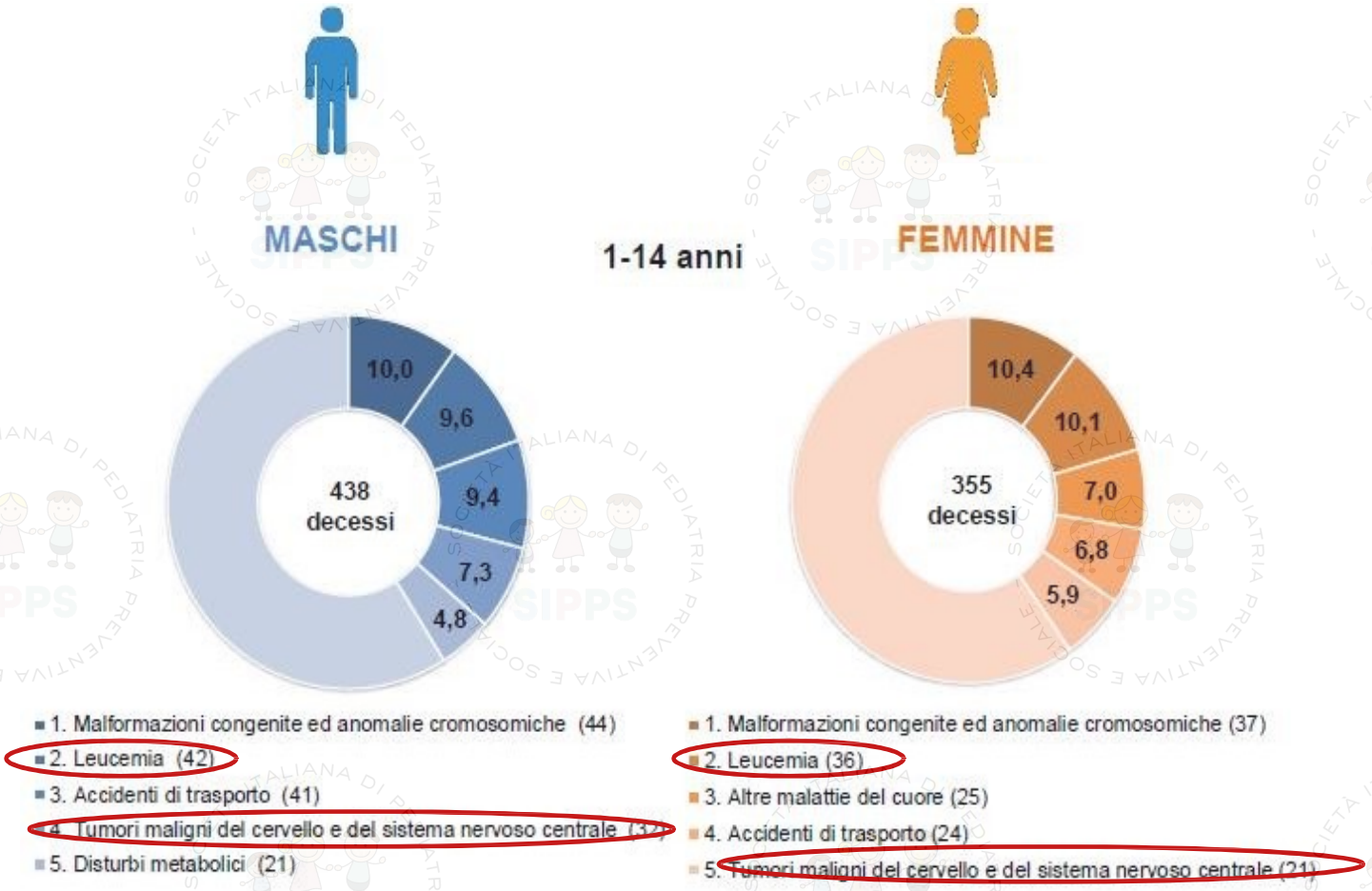
Tumori in età pediatrica: la "dimensione" del problema

- Patologia rara con incidenza di 15 nuovi casi/100.000 bambini compresi tra 0 e 14 aa/anno
- Ogni anno in Italia vengono diagnosticati circa 1400 nuovi casi di tumore tra i bambini
- La possibilità di ammalarsi di tumore in età pediatrica è 1: 600
- Un pediatra diagnostica nella sua vita professionale 1-5 casi di tumori maligni o di leucemie.

Rapporto AIRTUM 2012 • TUMORI INFANTILI*Epidemiol Prev 2013; 37 (1) suppl 1: 1-296*

Cause di morte

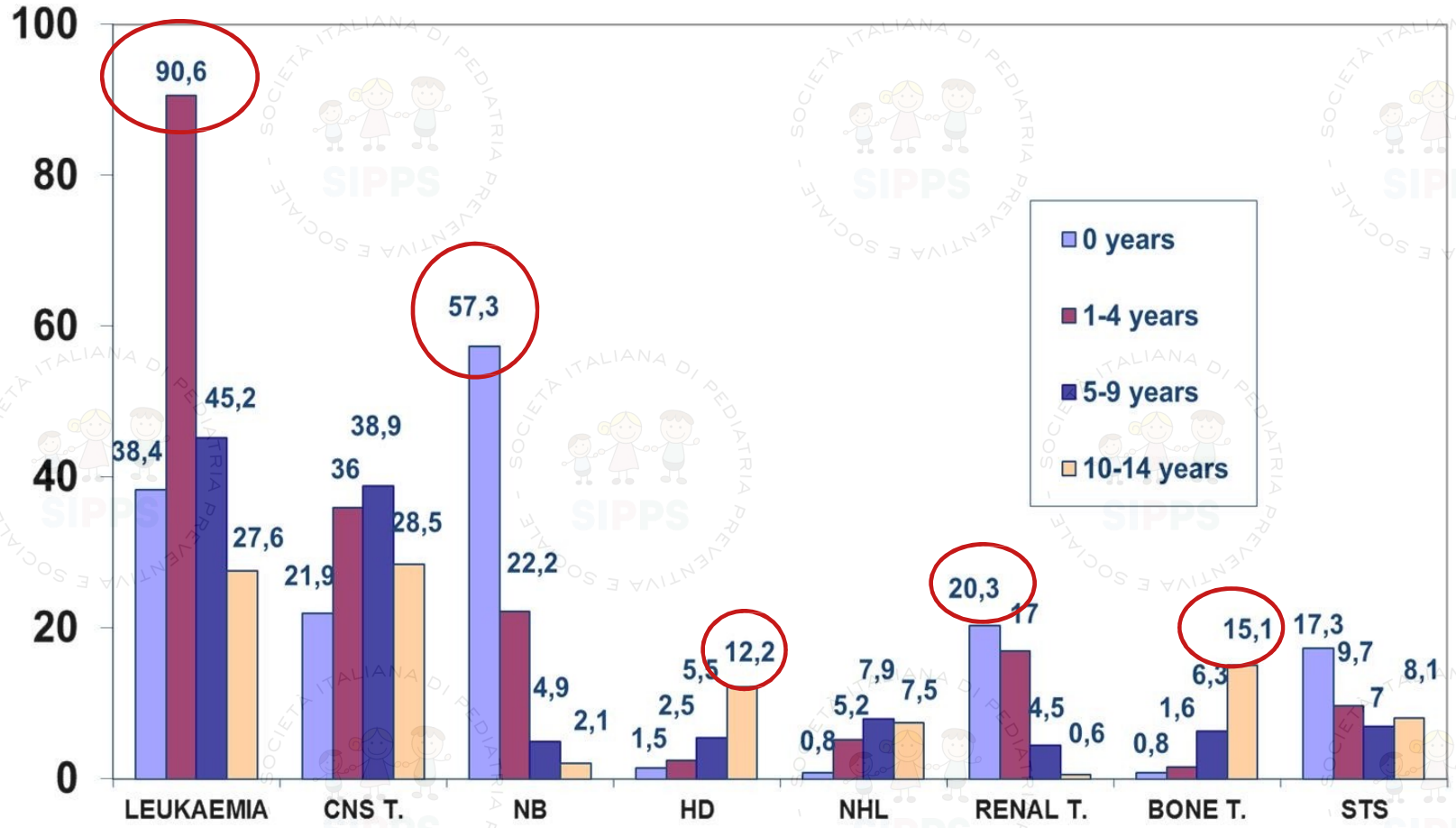


Istat 3 dicembre 2014

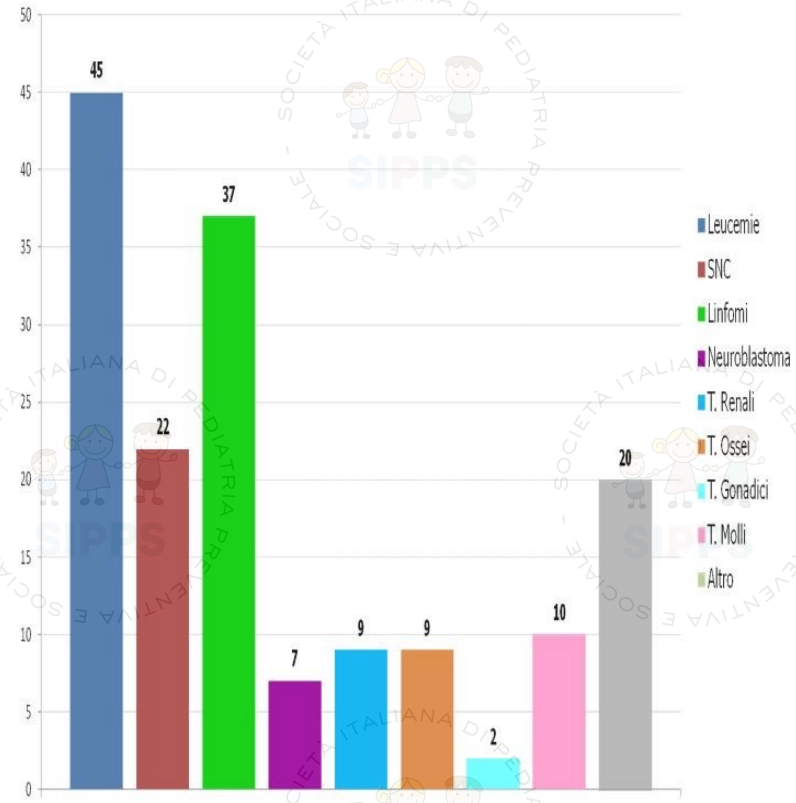
Diagnosi precoce: il problema

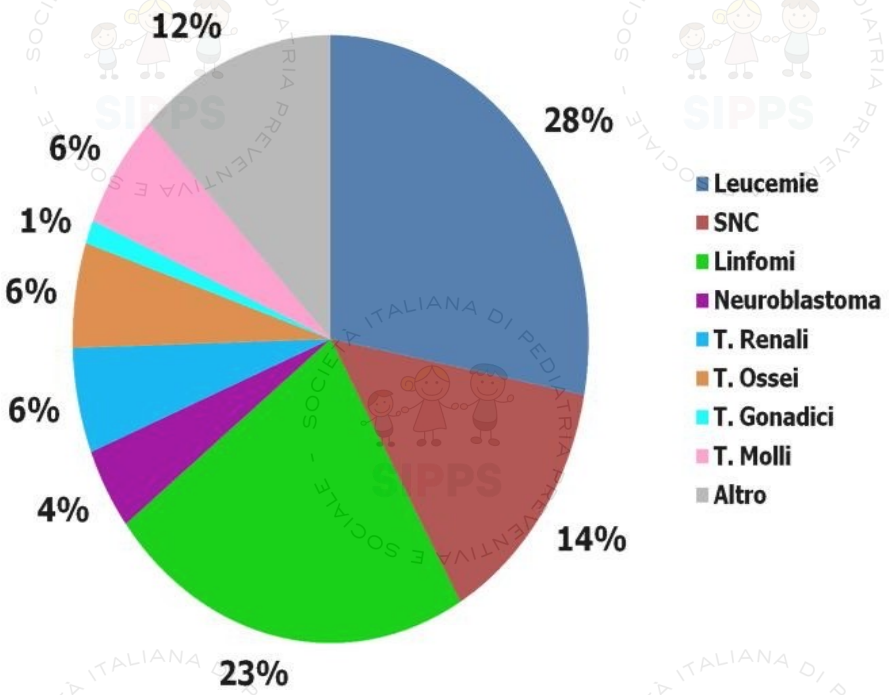
- Patologie poco frequenti
- Sintomi e segni clinici relativamente non specifici e spesso riportabili ad altre patologie più comuni e meno gravi
- Disponibilità di pochi markers tumorali
- Impossibilità di effettuare screening

Tumori ed età del bambino



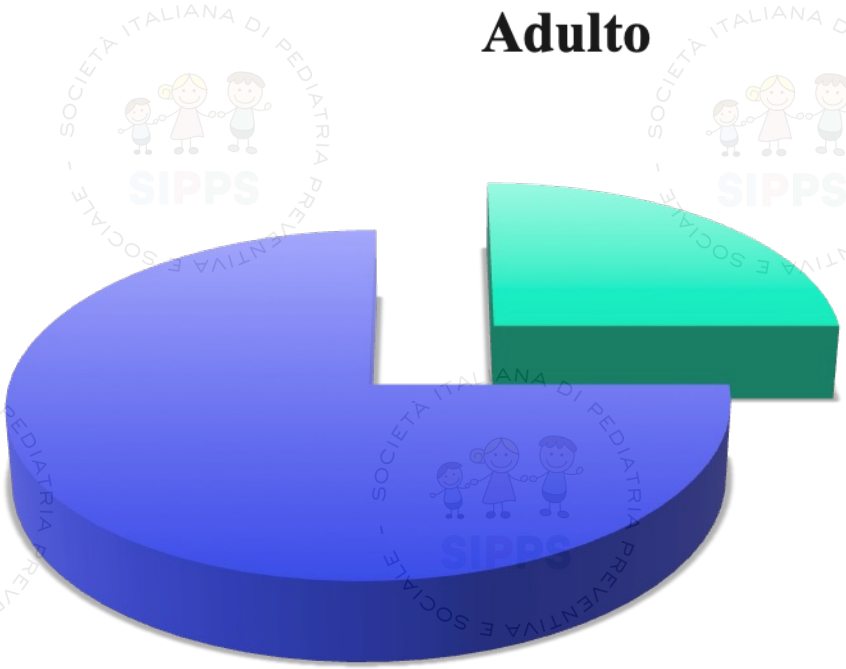
Tumori pediatrici in Campania (2013-2014)



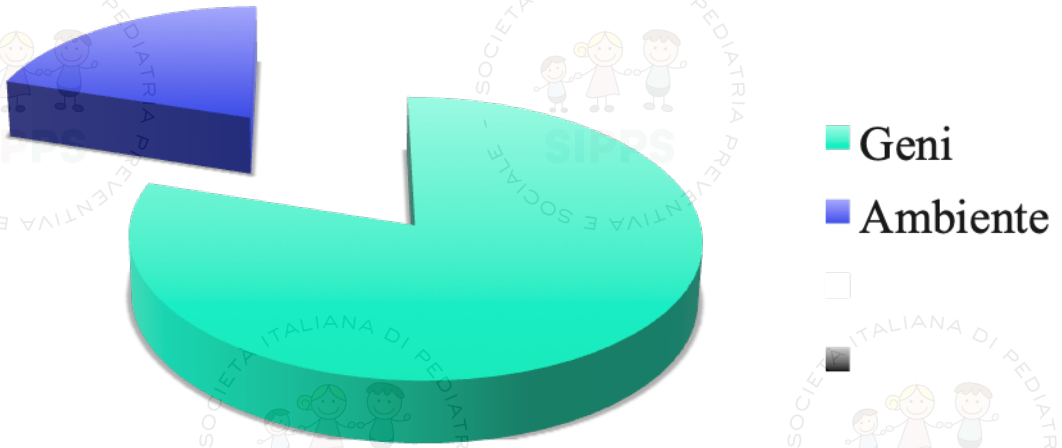


Differences in ped/adult cancers

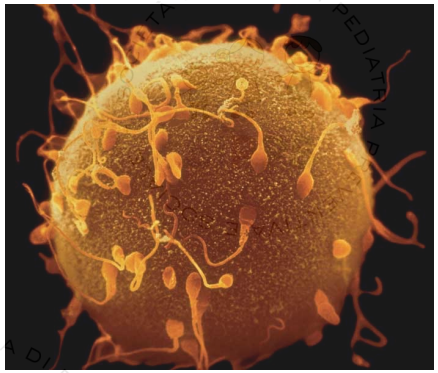
- **ADULT CANCERS** not seen in children:
  - Breast ca
  - Lung ca
  - Colon ca
  - Testicular ca
  - Prostate ca
- **PEDIATRIC CANCERS** not seen in adults:
  - Wilms tumor
  - Rhabdomyosarcoma
  - Neuroblastoma



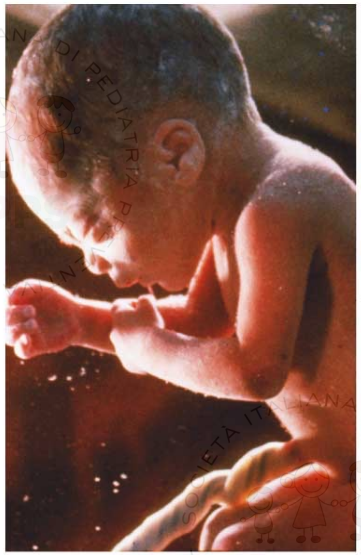
I tumori sono sempre dovuti al contributo di più fattori

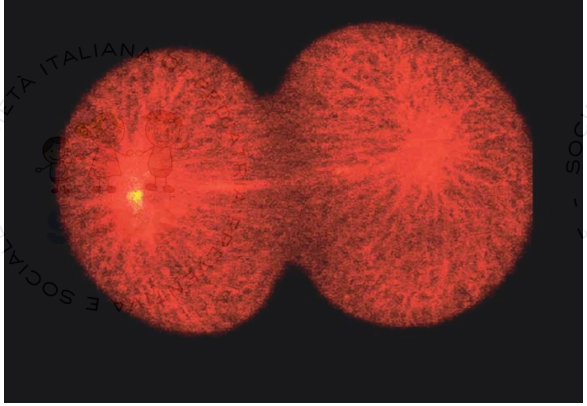


Sexual Reproduction



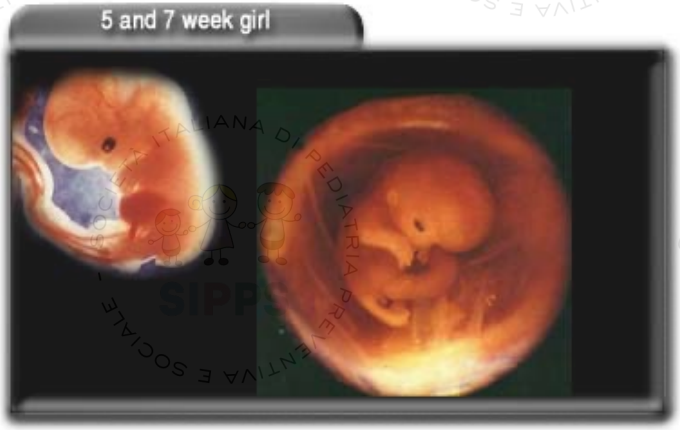
Once an egg becomes fertilized, cellular divisions begins, eventually producing a whole organism

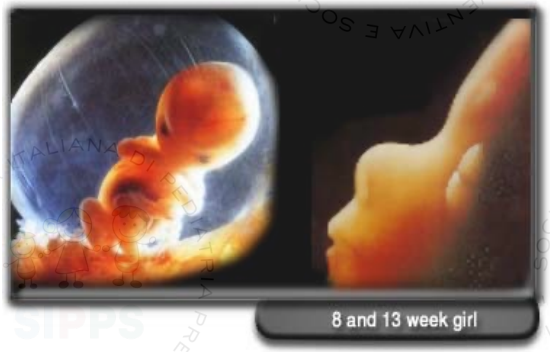




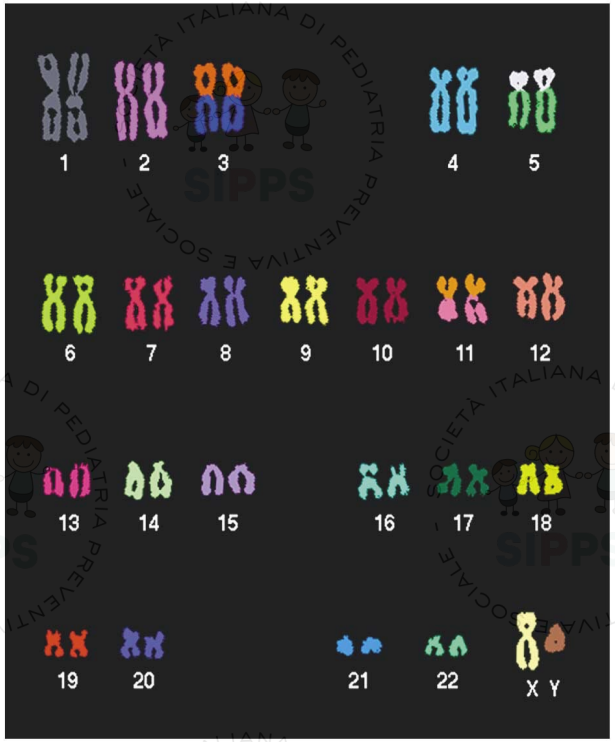
Malignancy is a loss of cell cycle coordination

All cells derived from the zygote contain the exact genetic material





REPLICATION>>>> DIFFERENTIATION

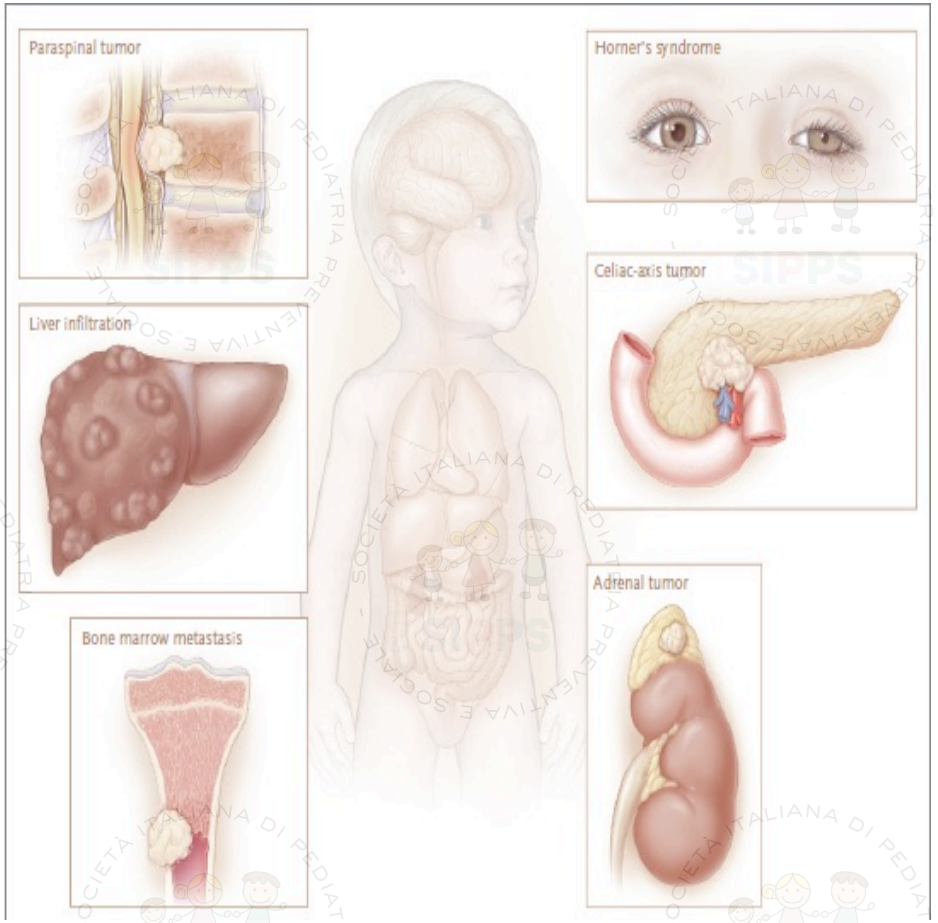


Domande in cerca di risposta:

-Siamo predisposti?
-Si può prevedere la risposta alla terapia?
-Si puo prevedere la ricaduta?
-Trovare l'alterazione sul DNA può servire a curare il tumore?
-Se si, come?

Neuroblastoma (NBL)

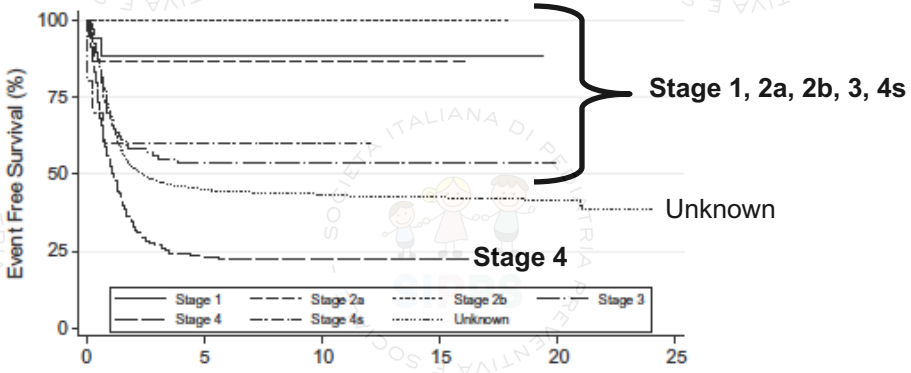
- Tumors can arise anywhere along the sympathetic nervous system, with the majority occurring in the adrenal medulla
- No proven environmental risk factors
- Median age dx = 17 months
- Remarkable clinical heterogeneity (Stage 4 and 4s)
- Sporadic and Familial (1%) Neuroblastoma



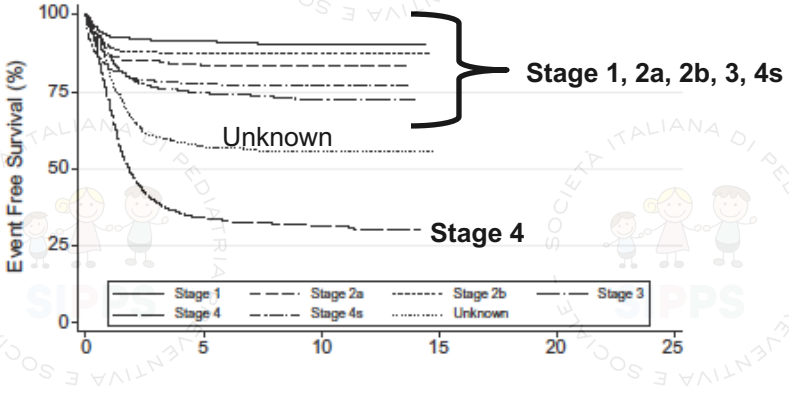
N ENGL J MED 362;23 NEJM.ORG JUNE 10, 2010

Stage 4 patients still have very low rate of event free survival

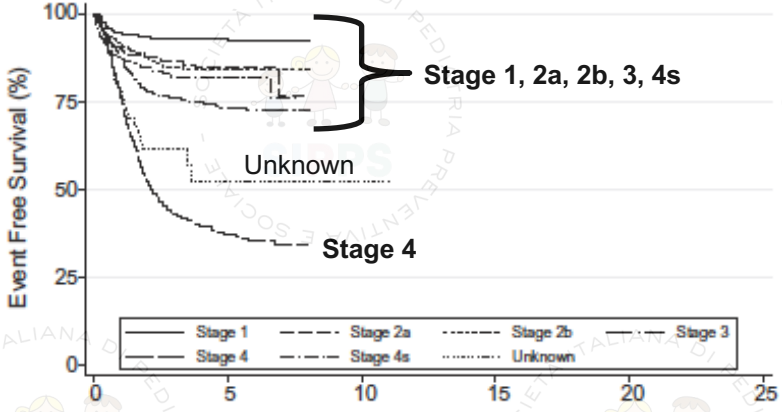
Era I: 1974-1989



Era II: 1990-1996



Era III: 1997-2002



Data from 11,307 children with neuroblastoma (1974-2002)

Moroz V et al., European Journal of Cancer, 2010

Genomics discoveries in neuroblastoma

1977-1989

- Brodeur et al 1977: ***1p deletion***
- Biedler et al, 1980: ***17q gain***
- Shawb et al 1983: ***MYCN ampl***
- Look et al 1984 : ***DNA diploid and high stage tumor***

1990-1999

- Svrivastan et al. 1993: ***11q deletion***
- Nakagawara et al. 1993: ***low expression TrkA***

2000-2009

- Trochet et al. 2004: ***PHOX2B mutations in familial neuroblastoma***
- Mosse et al. 2008: ***ALK mutations in familial and sporadic neuroblastoma***
- Maris et al. 2008: ***CASC15 susceptibility locus***
- Capasso et al. 2009: ***BARD1 susceptibility gene***

2010-present

- Versteeg et al. 2010: ***ATRX somatic mutations***
- Diskin, Capasso et al.2012: ***LIN28B susceptibility gene***
- Diskin, Capasso et al. 2014: ***TP53 susceptibility gene***
- ***Capasso et al. 2014 NEFL susceptibility gene***

The International Neuroblastoma Risk Group (INRG) Classification System

Poor prognosis:

Genetic Markers

DNA di-*ploidy*

MYCN amplification

Deletion 11q

17q gain and 1p36 deletion

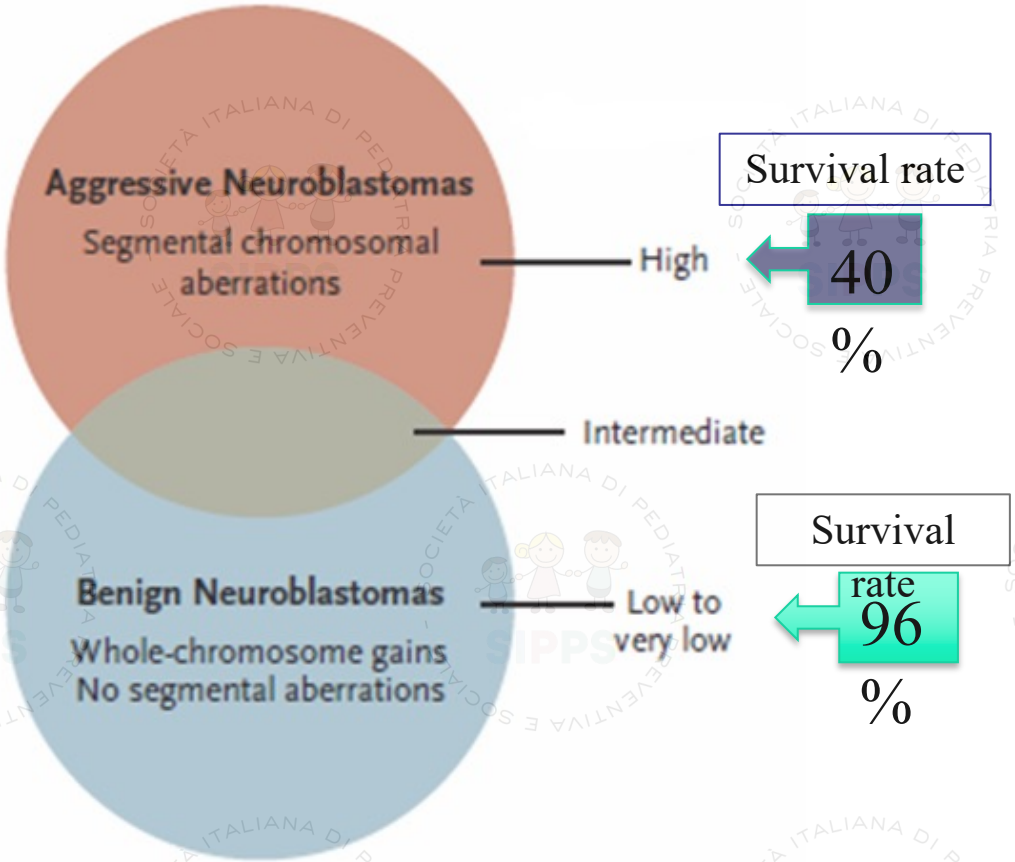
ALK mutations

BARD1 polymorphisms

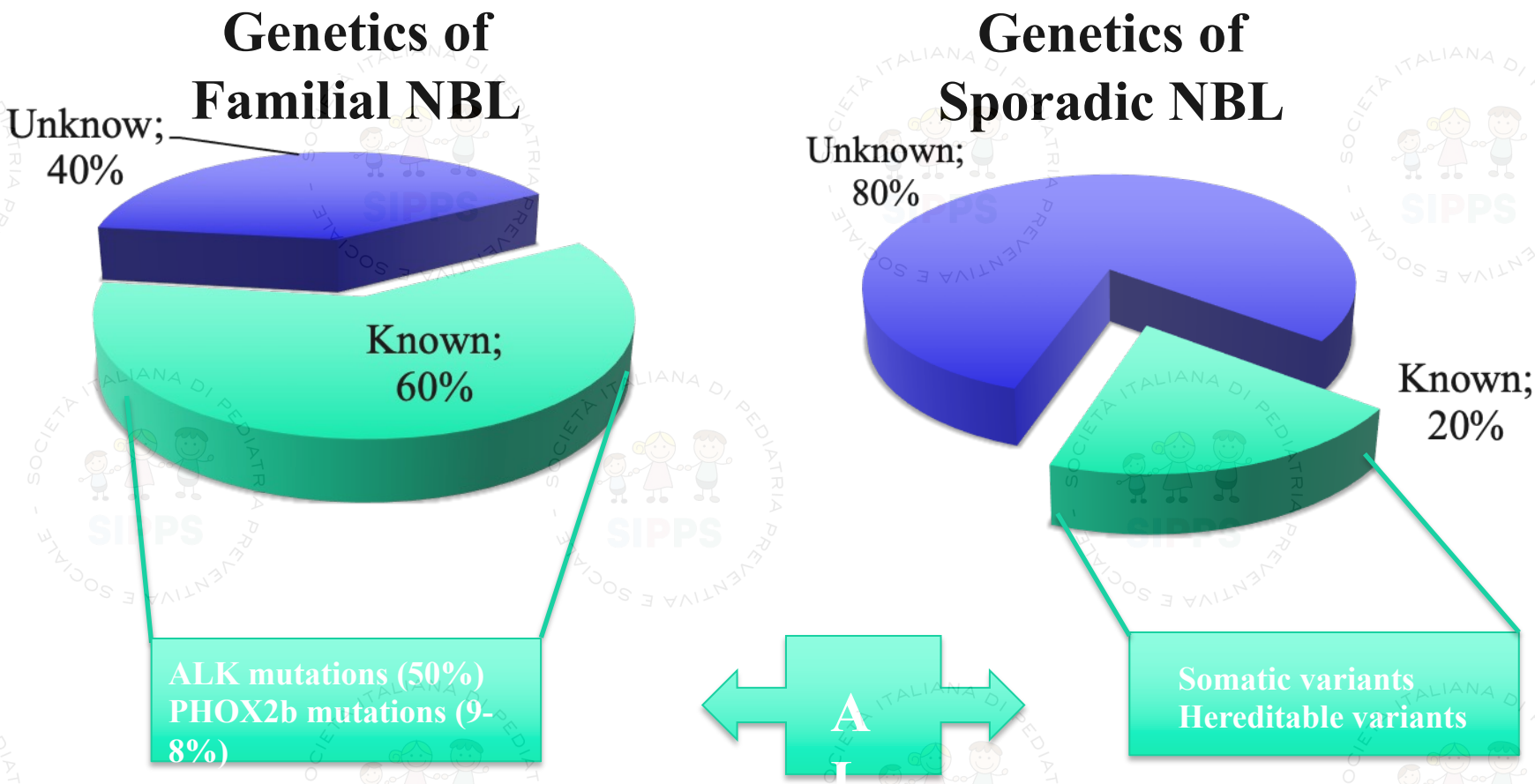
Clinical Markers

Age>18 months

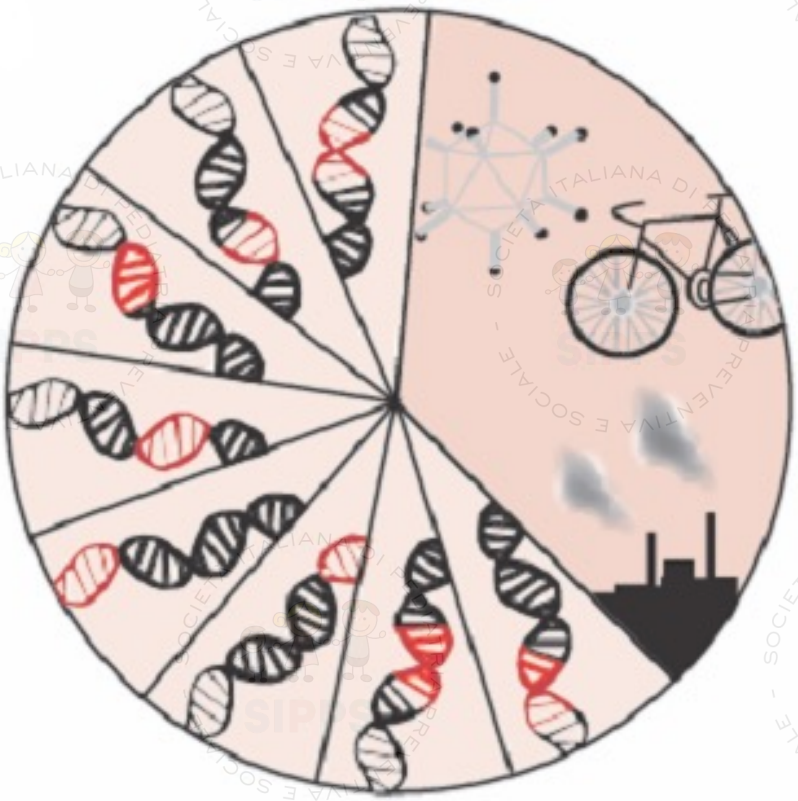
INSS stage = 4



Familial vs Sporadic Neuroblastoma



Complex disease

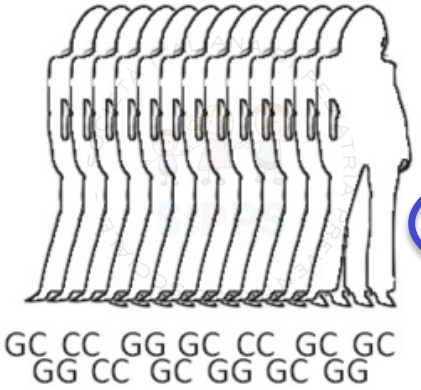


Complex disease: many variants of small effect contribute to disease risk, along with many environmental factors.

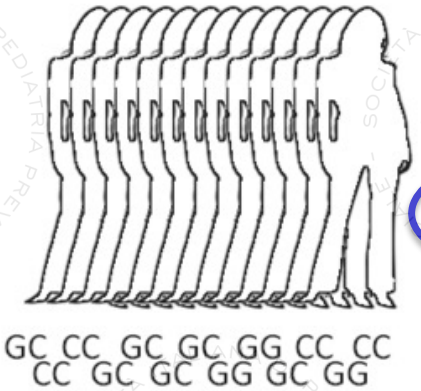
GWAS analysis:

Thousands of cases and controls and 1 million of SNPs

Cases



Controls

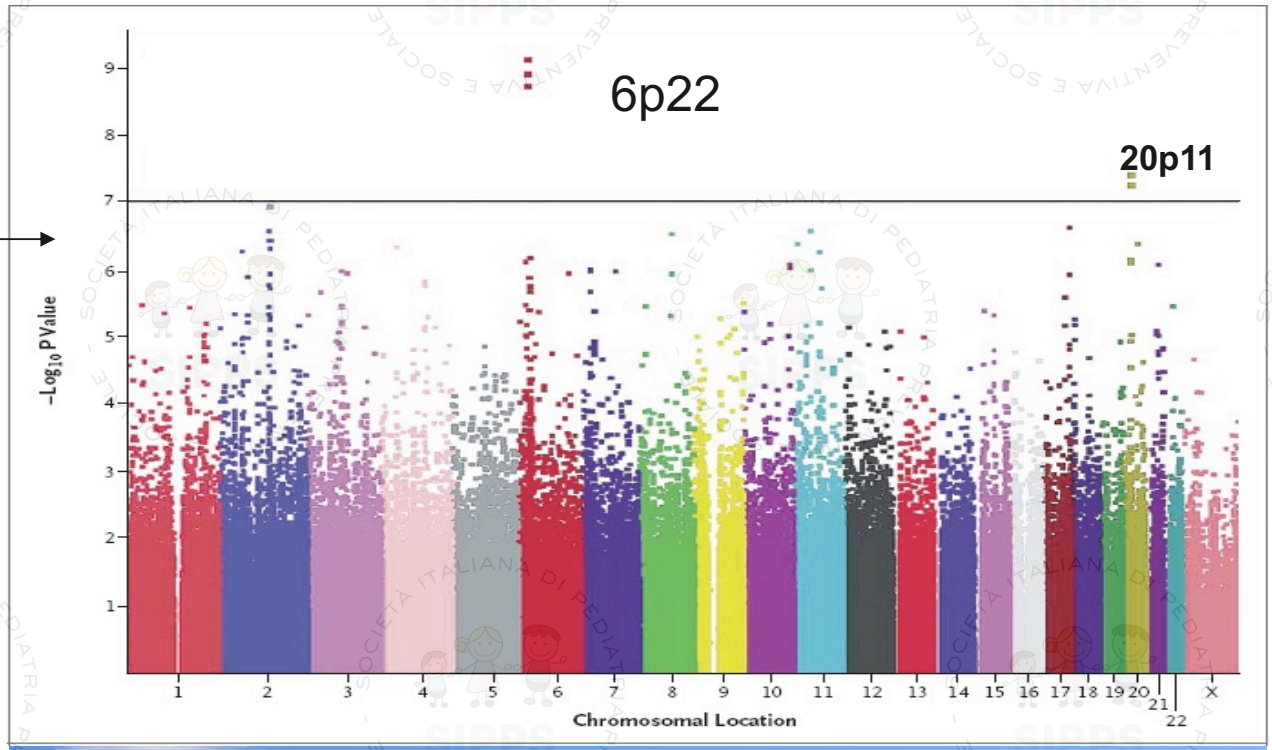


SNP1	*SNP2*	*SNP …*
Cases Count of G: 2104 of 4000	**Cases** Count of G: 1648 of 4000	*Repeat for all SNPs*
Frequency of G: 52.6%	Frequency of G: 41.2%	
Controls Count of G: 2676 of 6000	**Controls** Count of G: 2532 of 6000	
Frequency of G: 44.6%	Frequency of G: 42.2%	
P-value: $5.0 \cdot 10^{-15}$	**P-value:** 0.33	

P-value<$5x10^{-8}$

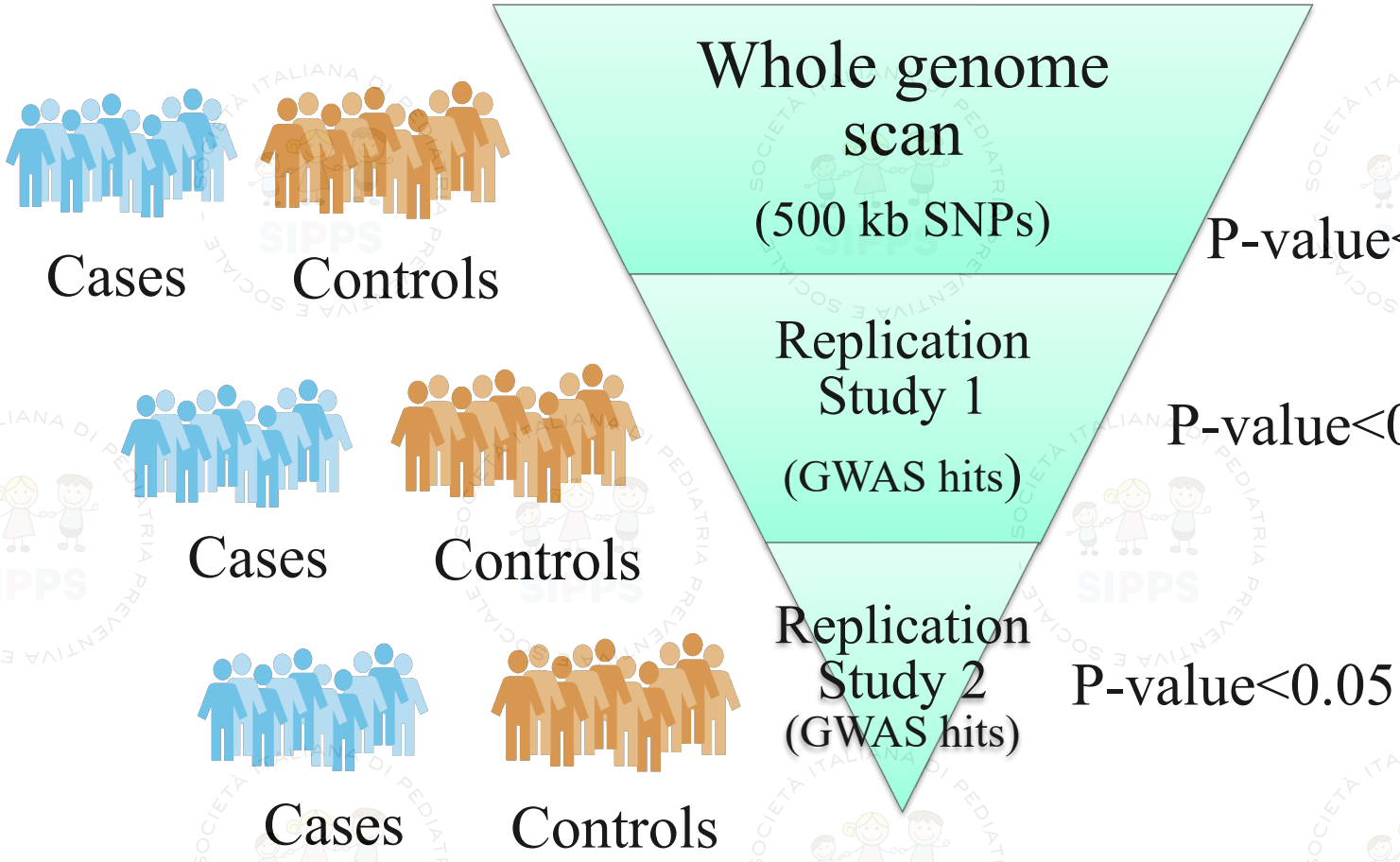
Manhattan plot

Genome-wide significance threshold



Maris et al, NEJM 2008

GWAS: Replication Study approach



Real SNP-associations

Trait-associated SNPs are often located in gene regulatory regions

- Top-scoring GWAS SNPs are frequently positioned within enhancer elements specifically active in relevant cell types (Ernst, Nature 2011)

a

Phenotype	Top cell type	Total no. SNPs from study	No. SNPs in enh. states 4 and 5	*P* value	FDR	H1 ES	K562	GM12878	HepG2	HUVEC	HSMM	NHLF	NHEK	HMEC
Erythrocyte phenotypes	K562	35	9	$<10^{-7}$	0.02	9	17	4	0	0	1	2	1	1
Blood lipids	HepG2	101	13	$<10^{-7}$	0.02	3	5	0	11	2	3	3	4	3
Rheumatoid arthritis	GM12878	29	7	2.0×10^{-7}	0.03	0	0	15	0	2	0	0	2	3
Primary biliary cirrhosis	GM12878	6	4	6.0×10^{-7}	0.03	0	11	41	0	0	0	0	8	8
Systemic lupus erythematosus	GM12878	18	6	9.0×10^{-7}	0.03	0	4	21	0	5	8	0	3	5
Lipoprotein cholesterol/triglycerides	HepG2	18	5	1.2×10^{-6}	0.03	17	8	0	24	3	6	4	3	3
Haematological traits	K562	39	7	1.7×10^{-6}	0.03	0	12	10	2	1	0	0	1	0
Haematological parameters	K562	28	6	2.2×10^{-6}	0.03	0	15	7	0	5	7	7	3	2
Colorectal cancer	HepG2	4	3	3.8×10^{-6}	0.03	0	0	0	66	0	12	0	12	12
Blood pressure	K562	9	4	5.0×10^{-6}	0.04	0	30	14	0	10	6	7	5	11

GWAS of neuroblastoma 2007

The Children's Hospital of Philadelphia

Università di Napoli "Federico II" DMMBM- CEINGE

Genetic Etiology of NBL

- 99% of cases sporadic
  - Genetic etiology not known

Hypothesis: Neuroblastoma is a complex genetic disease that results from the interaction of variant alleles with relatively low to moderate effect on tumor initiation

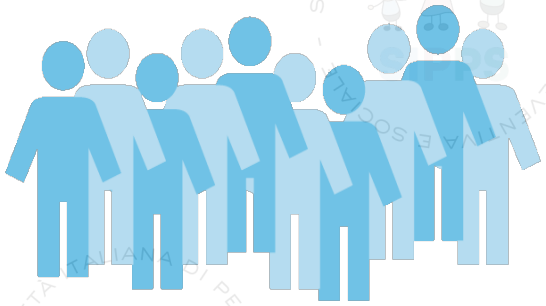
Experiment: Genome-wide association study comparing neuroblastoma cases to children without cancer as controls

GWAS of neuroblastoma

USA

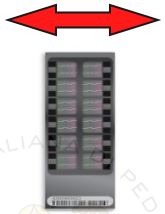
Cases

Children affected by neuroblstoma



5.000 constitutional DNAs

vs.



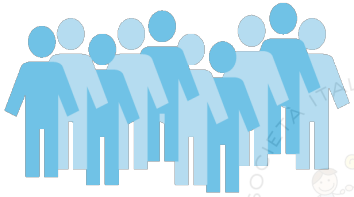
Illumina CHIP

Controls

Healthy subjects

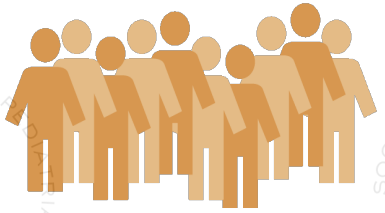
10.000 constitutional DNAs

ITA



500 constitutional DNAs

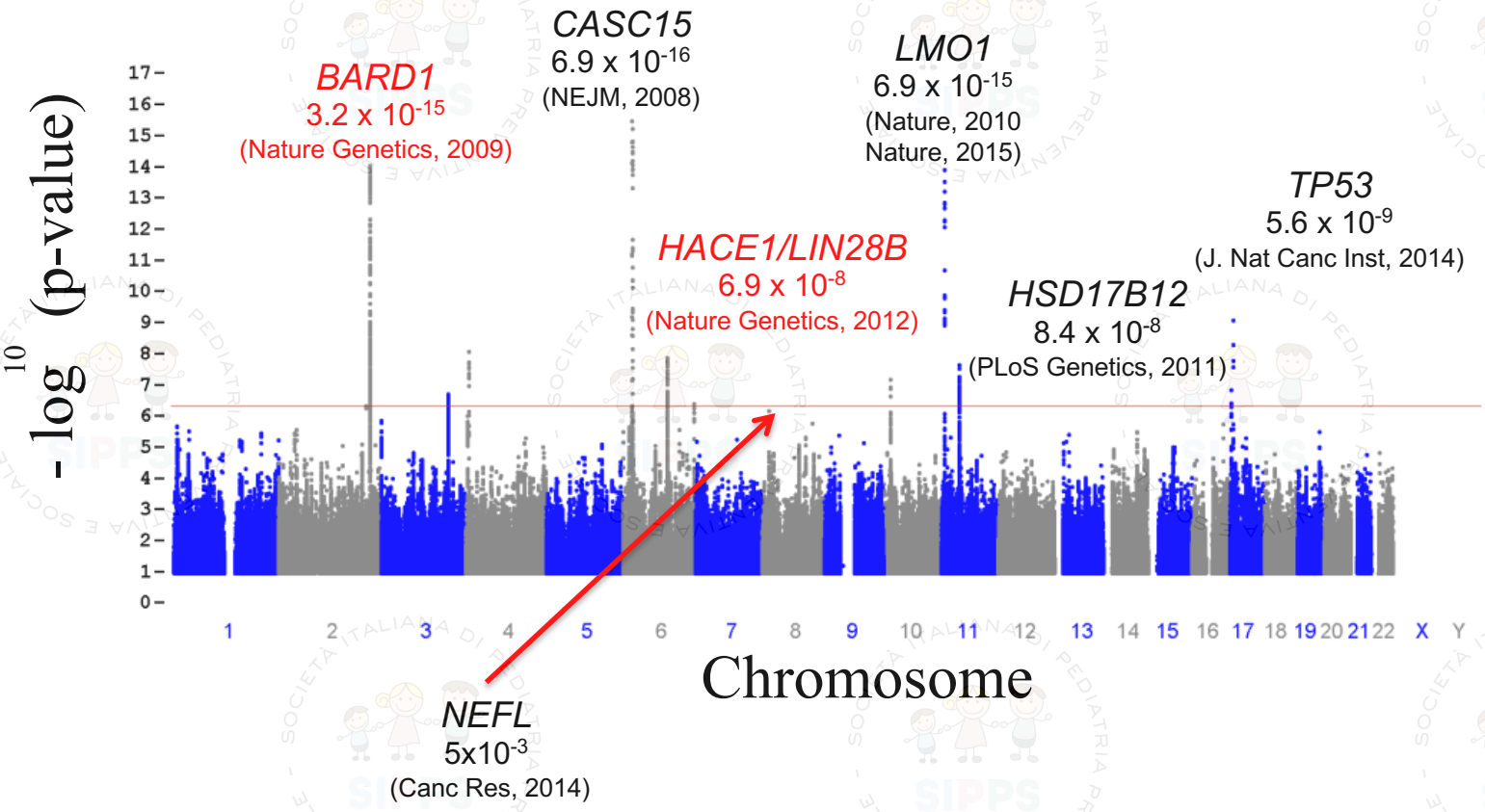
RealTime PCR genotyping



1000 constitutional DNAs

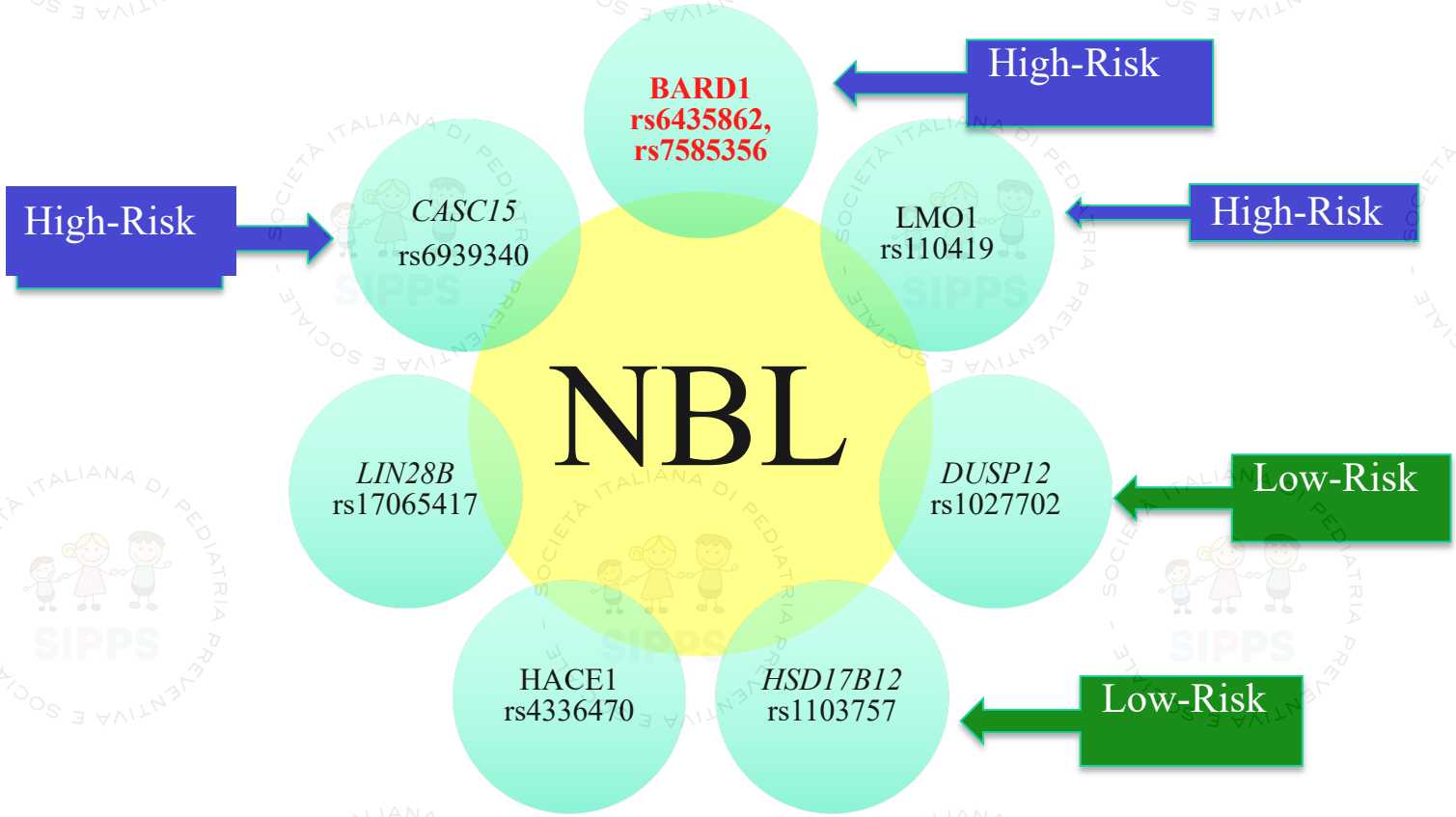
Neuroblastoma susceptibility loci

2,101 cases and 4,202 genetically matched controls (European ancestry)



Carcinogenesis vol.00 no.00 p.1 of 7, 2012
doi:10.1093/carcin/bgs380
Advance Access publication December 7, 2012

Replication of GWAS-identified neuroblastoma risk loci strengthens the role of *BARD1* and affirms the cumulative effect of genetic variations on disease susceptibility

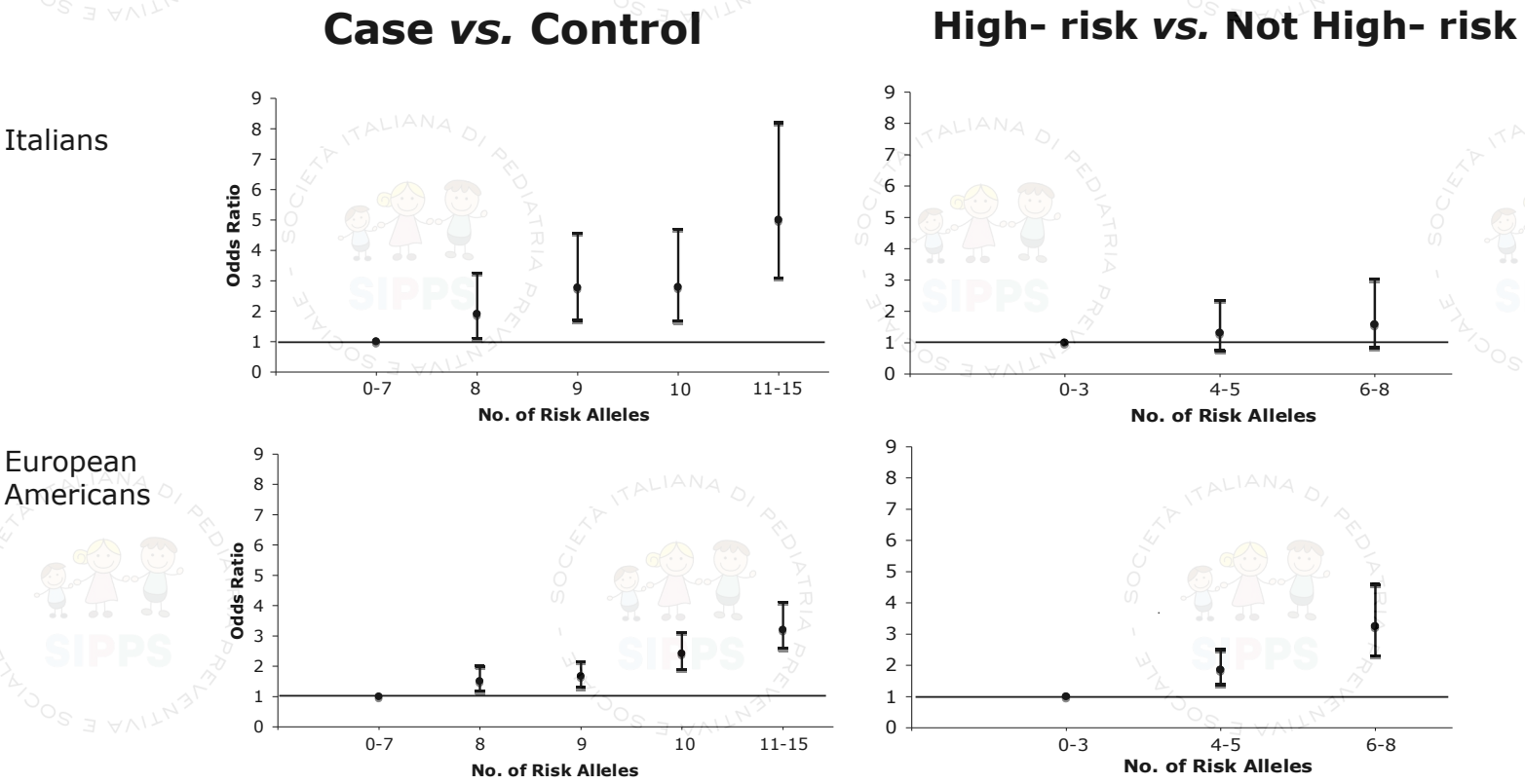


7 independent NB-associated SNPs robustly validated in Italian population

350 cases and 800 controls

Capasso et al., 2013 Carcinogenesis

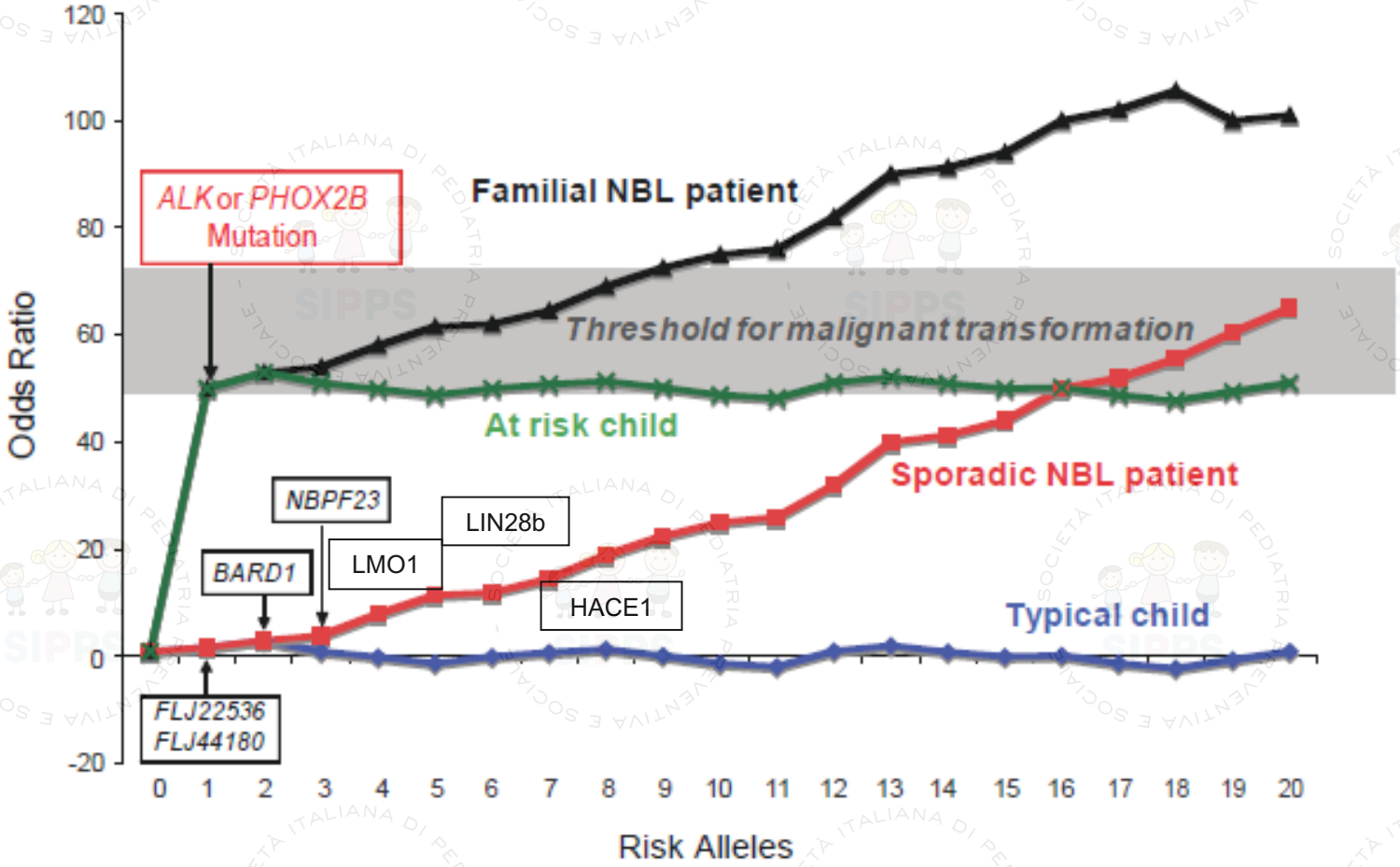
Cumulative effect of genetic variations on disease susceptibility



The risk of neuroblastoma increases with increasing of number of risk genetic variants

Capasso et al., 2013 Carcinogenesis

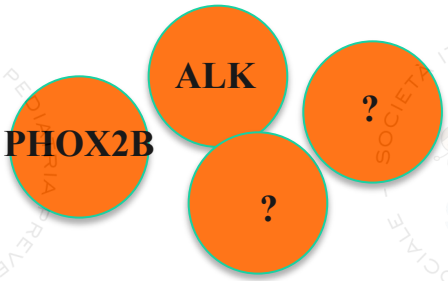
Genetic Model of Sporadic Neuroblastoma

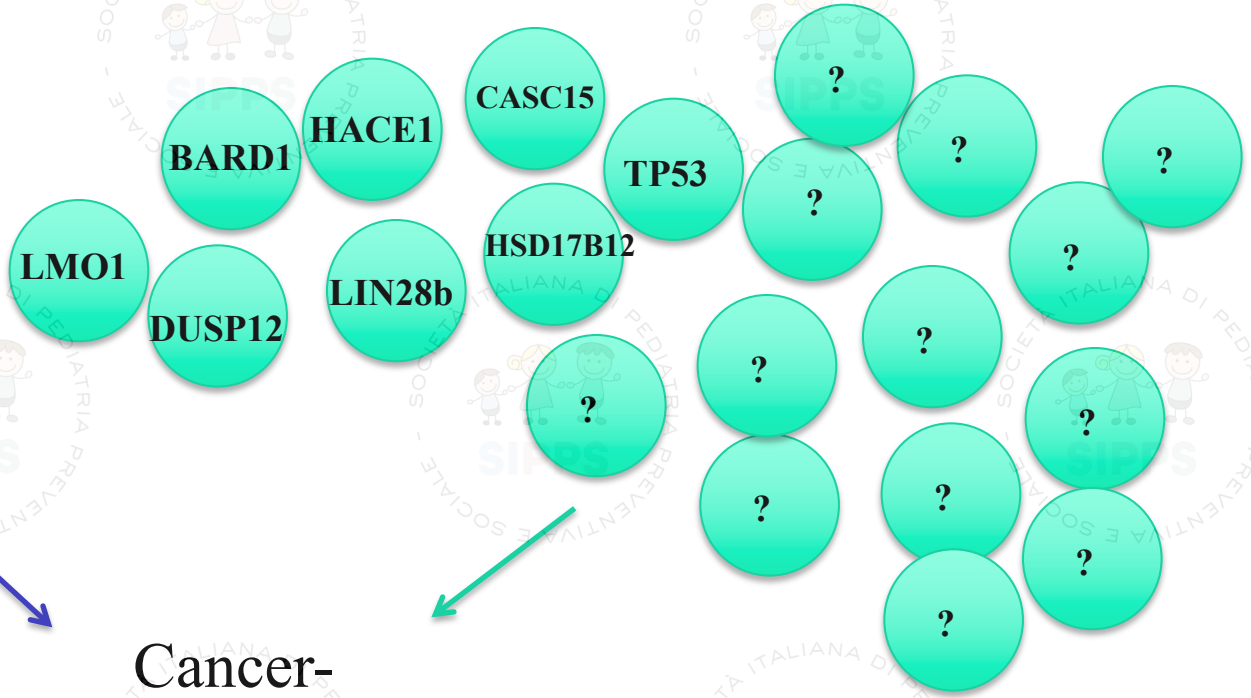


Capasso M, Diskin SJ. Cancer Treat Res. 2010

Sporadic NB: **POLYGENIC INHERITANCE**

Familial NB: **MONOGENIC DEFECTS**





Environmental factors

Cancer-prone condition

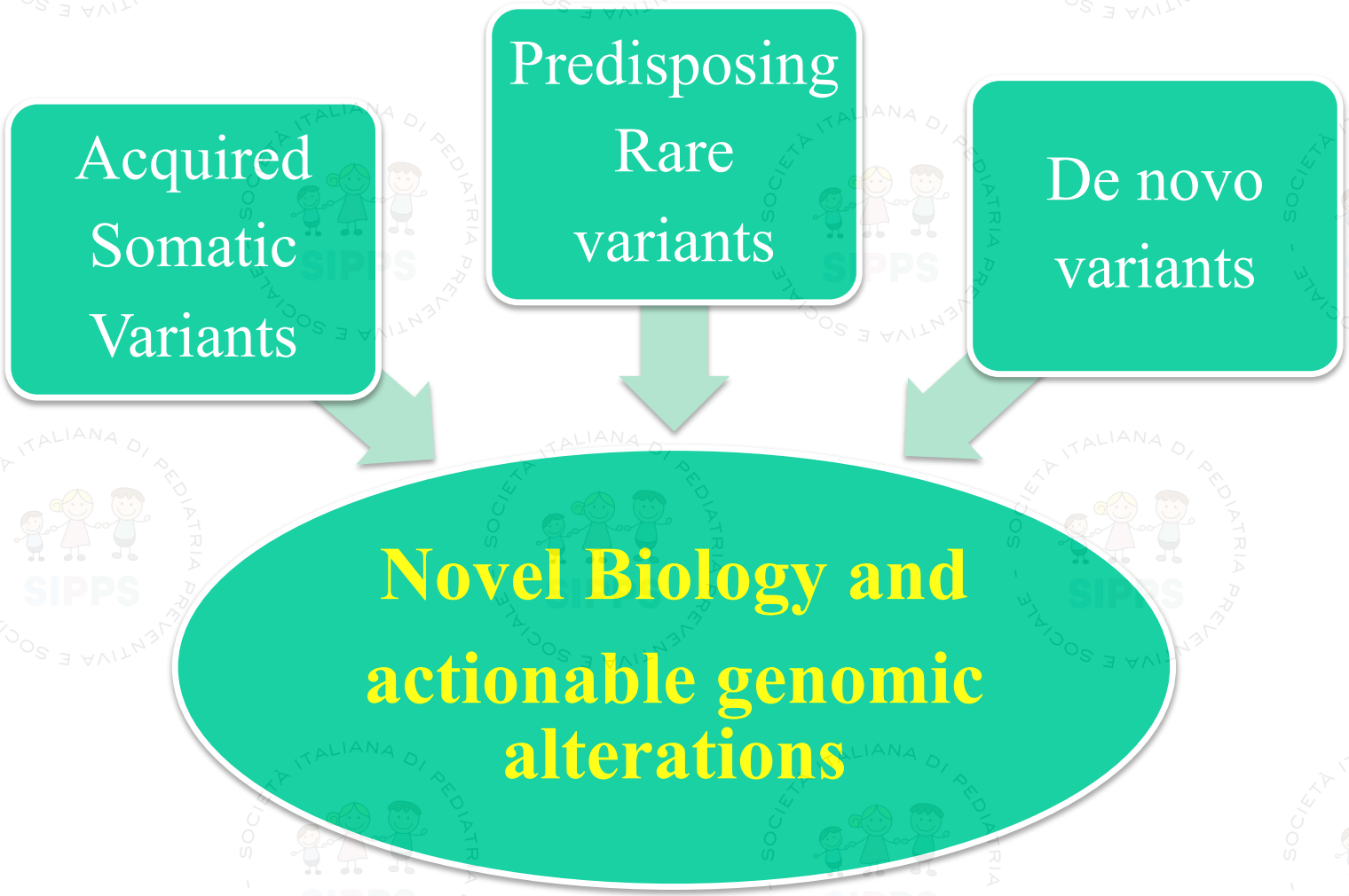
↓

Somatic Mutations

↓

Neuroblastoma

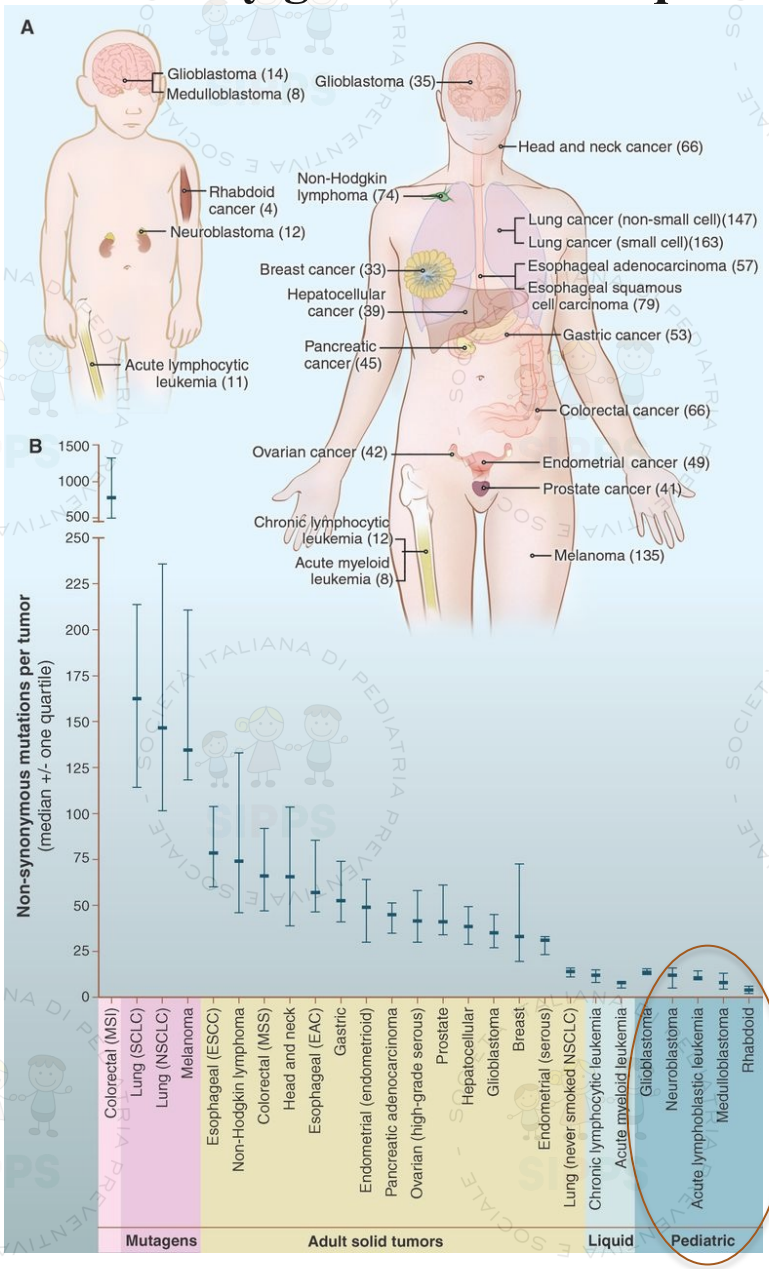
Next Generation Sequencing of neuroblastoma



Number of somatic mutations in representative human cancers, detected by genome-wide sequencing studies

In pediatric tumors:

1. **Low rate mutations**
2. **Only genes, frequently mutated, have been discovered**
3. **Larger role of germline variants in cancer pediatric**



Bert Vogelstein, Science 2013

NGS studies of primary Neuroblastoma demonstrate

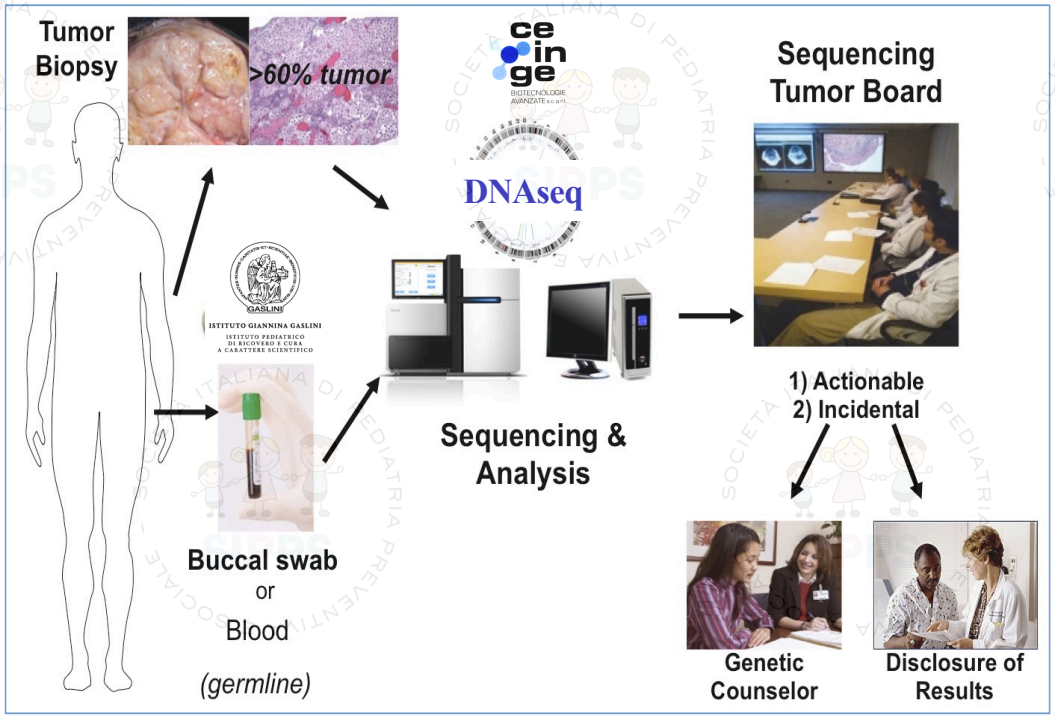
- Low rate of somatic mutations
- Few genes frequently mutated (10-20%) across patients

PREME

Exome Sequencing of relapsed/high-risk neuroblastoma

Find patients eligible for novel treatments based on the somatic mutation

Molecular Tumor Board
- oncologists
- genetics
- pathologists
- biologists
- bioinformaticians
- genetic counselors



8 ALK mutations in 27 relapsed/refractory NB

2018-2021

- Hot-spot mutation **F1174L** in 6 patients
- Less frequent mutation **R1275Q** in 1 patient
- Novel pathogenic mutation **S104R** in 1 patient

MTB recommendations were delivered in 43 days (range: 31 to 96 days), including the time for DNA preparation, for sequencing and bioinformatic analyses.

-5 patients received crizotinib (3 patients died before starting treatment) in combination with chemotherapy and 2 in monotherapy

-All patients showed a clinical improvement, and one had a CR after 2 cycles of combined treatment

PREME

Case 1

Published in final edited form as:
J Neurooncol. 2018 May ; 138(1): 199–207. doi:10.1007/s11060-018-2791-y.

Phase I Trial of Dasatinib, Lenalidomide, and Temozolomide in Children with Relapsed or Refractory Central Nervous System Tumors

- 3-year-old male with early relapsed NB
- G320C in CUL4A gene -> Lenalidomide mieloma multiplo, sindromi mielodisplasiche
- Lenalidomide at the dose of 25 mg daily for 21 days every 28 days together with temozolomide at the dose of 250 mg daily for 5 days every 28 days
- The patient experienced **good clinical conditions until the end of the 3th cycle** when a headache recurred . Gadolinium-enhanced brain MRI showed **disease progression**. Clinical conditions rapidly worsened, the patient did not respond to treatment and died.
- The combination of temozolomide and lenalidomide was well tolerated in term of hematologic toxicity.

Case 2

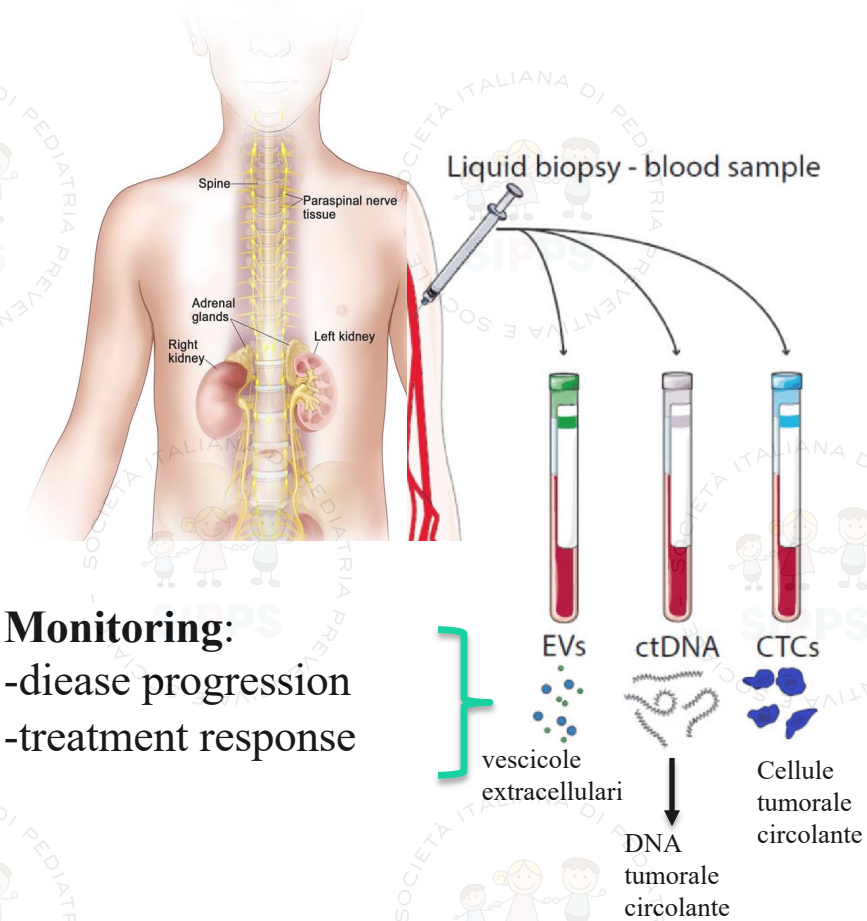
- 20 year-old male who relapsed 10 years after the first diagnosis of NB
- c.A484G in the PSMC2 gene ->Bortezomib (inhibitor of proteosome)
- Off label target therapy with 3 cycles of Bortezomib and Irinotecan and 2 cycles of TEMIRI with standard treatment
- Patient is in complete remission

Liquid Biopsy

Liquid biopsy for **label-free** detection of "**circulating tumor cells**" through digital **holographic microscopy** and **artificial intelligence**

Dr. P. Ferraro

Stain-free identification of cell nuclei using tomographic phase microscopy in flow cytometry



Monitoring:
-diease progression
-treatment response

A Targeted Gene Panel for Circulating Tumor DNA Sequencing in Neuroblastoma

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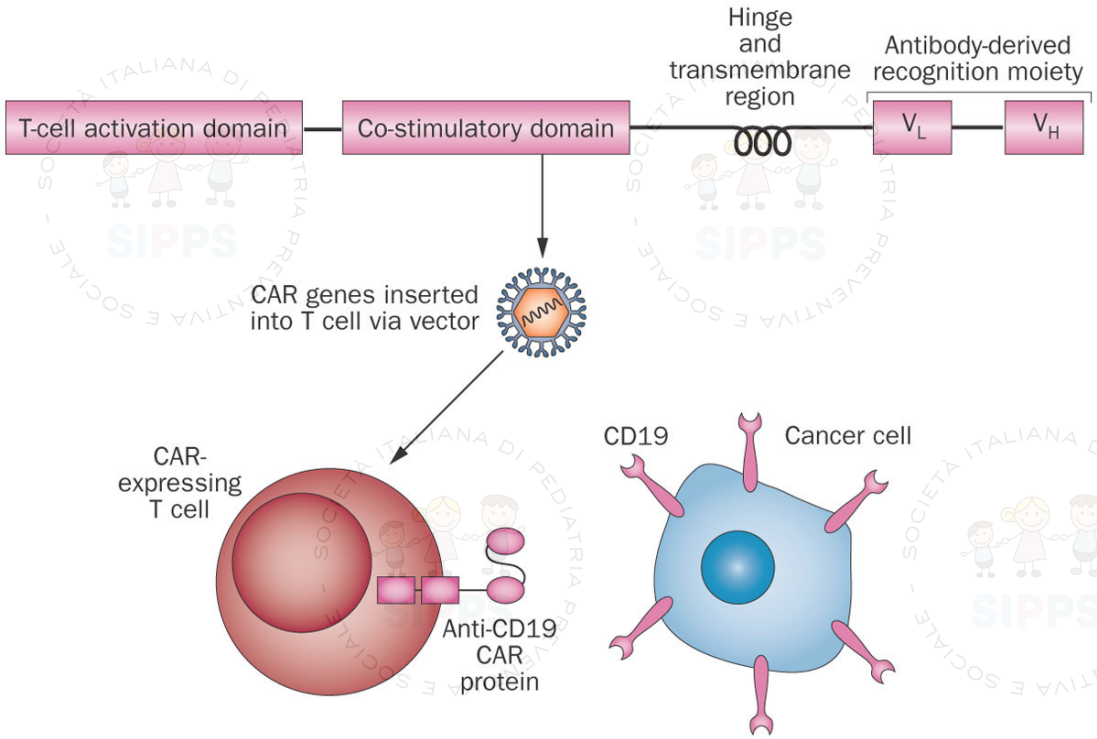
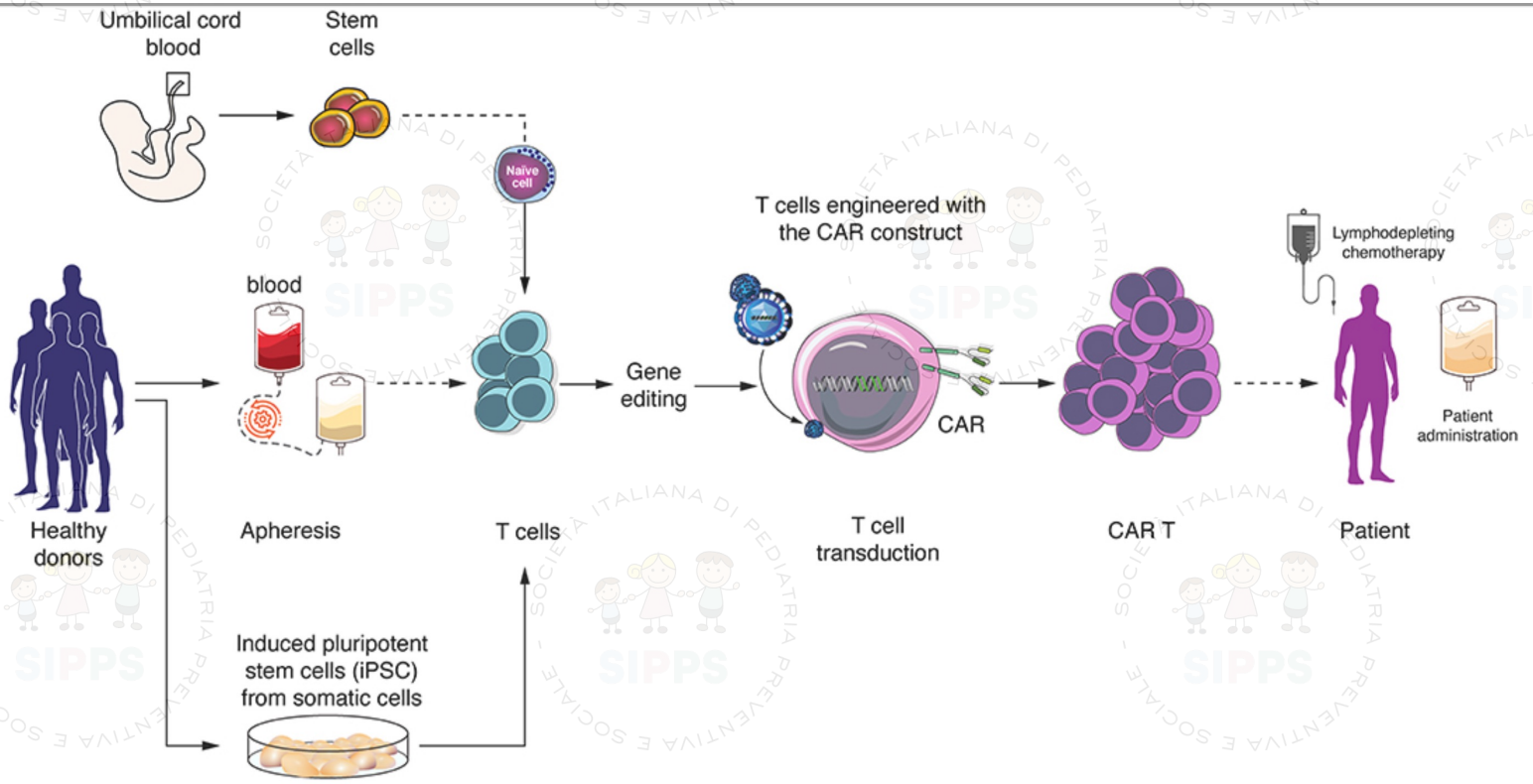
frontiers in Oncology

We developed a targeted NGS approach to identify tumor-specific alterations in ctDNA of neuroblastoma patients.

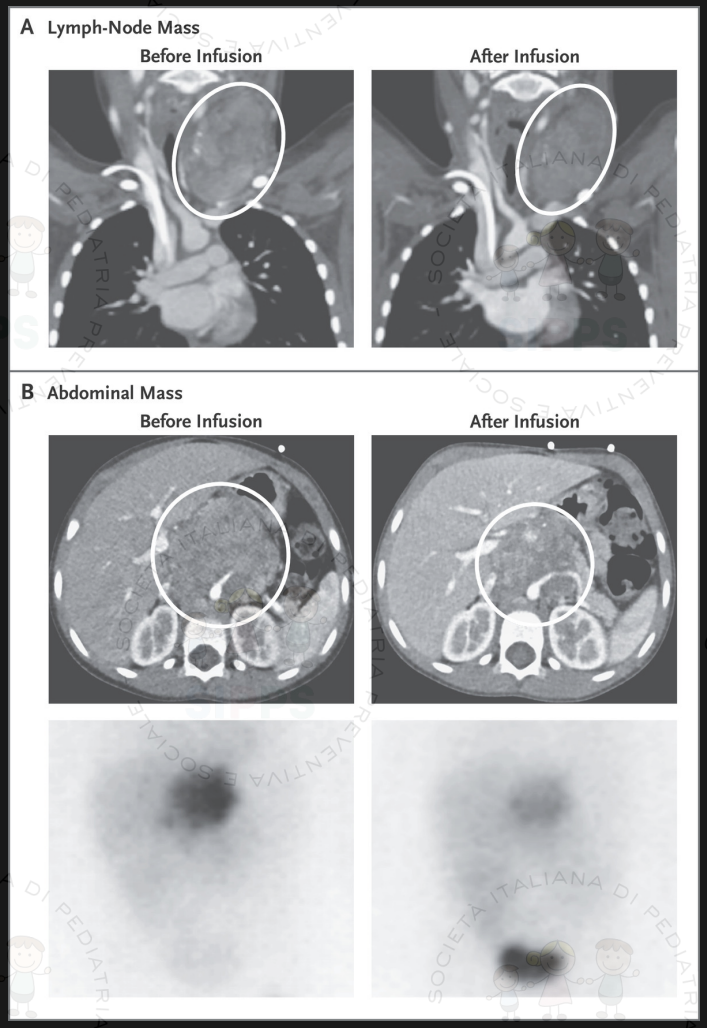
Chimeric Antigen Receptors (CARs)



Kochenderfer, J. N. & Rosenberg, S. A. (2013)
Nat. Rev. Clin. Oncol.


Umbilical cord blood
Stem cells
Naïve cell
T cells engineered with the CAR construct
Lymphodepleting chemotherapy
blood
Gene editing
CAR
Patient administration
Healthy donors
Apheresis
T cells
T cell transduction
CAR T
Patient
Induced pluripotent stem cells (iPSC) from somatic cells

Radiologic Response in Two Patients with Bulky Disease.



F Del Bufalo et al. N Engl J Med 2023;388:1284-1295.

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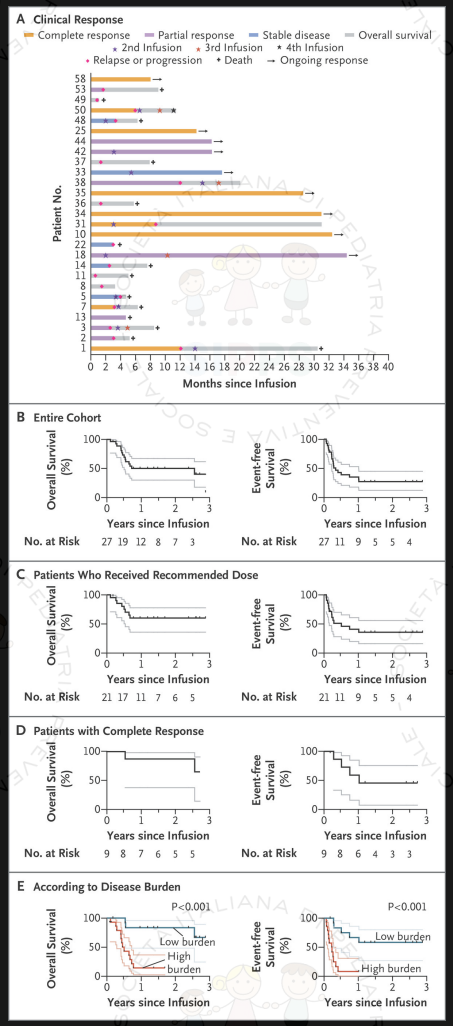
Long-Term Outcomes after GD2-CART01 Infusion.



Arruolati. 27 non-responders o recidivanti
DOPO 30 MESI
9 RISPOSTE COMPLETE
8 RISPOSTE PARZIALI
36% EVENT FREE SURVIVAL

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Lab group

Prof. Mario Capasso