Large-Scale Analysis of imprinting status in Pleuropulmonary blastoma cancer stem cells

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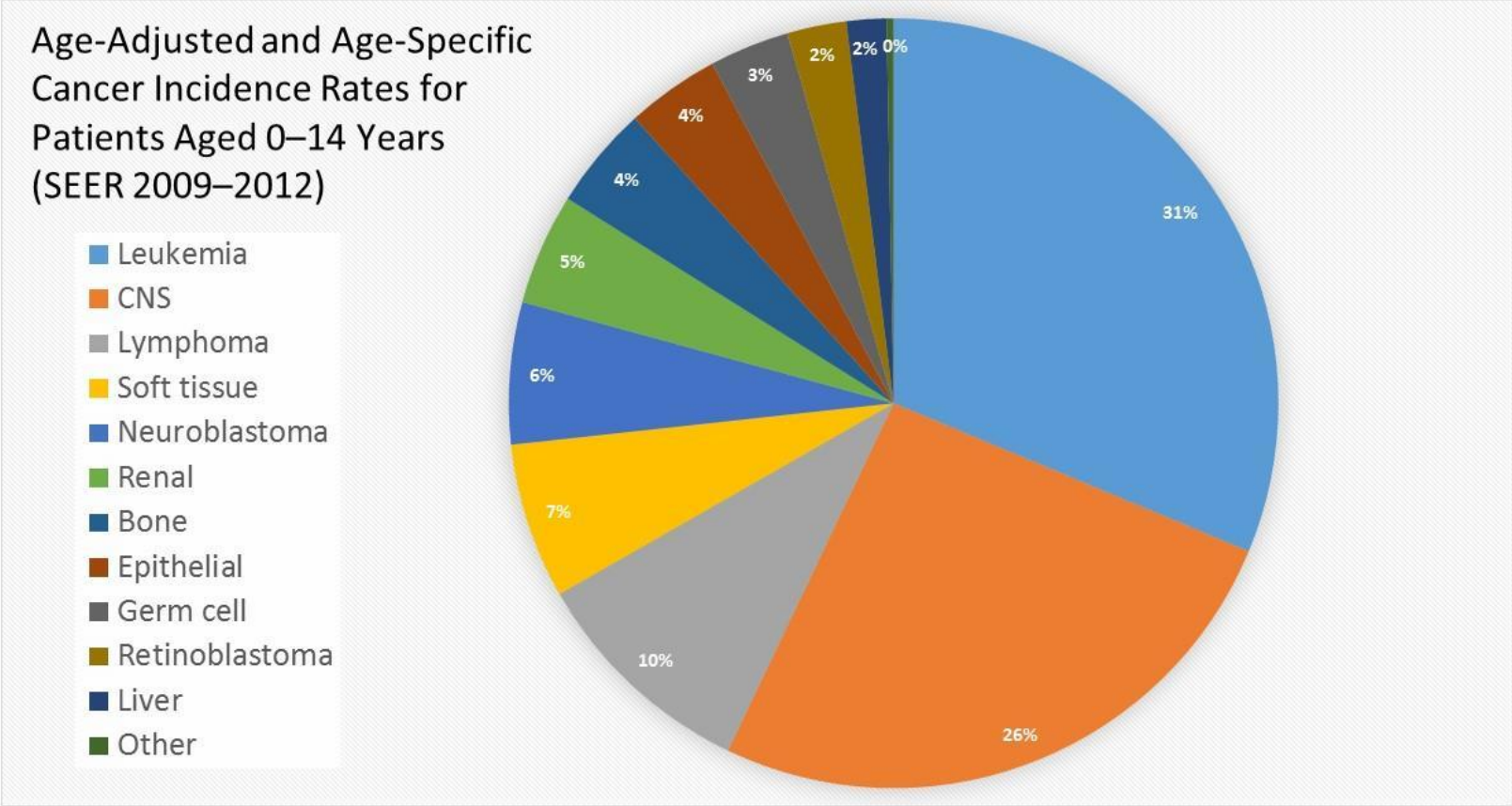
Bench To Bedside

Overview

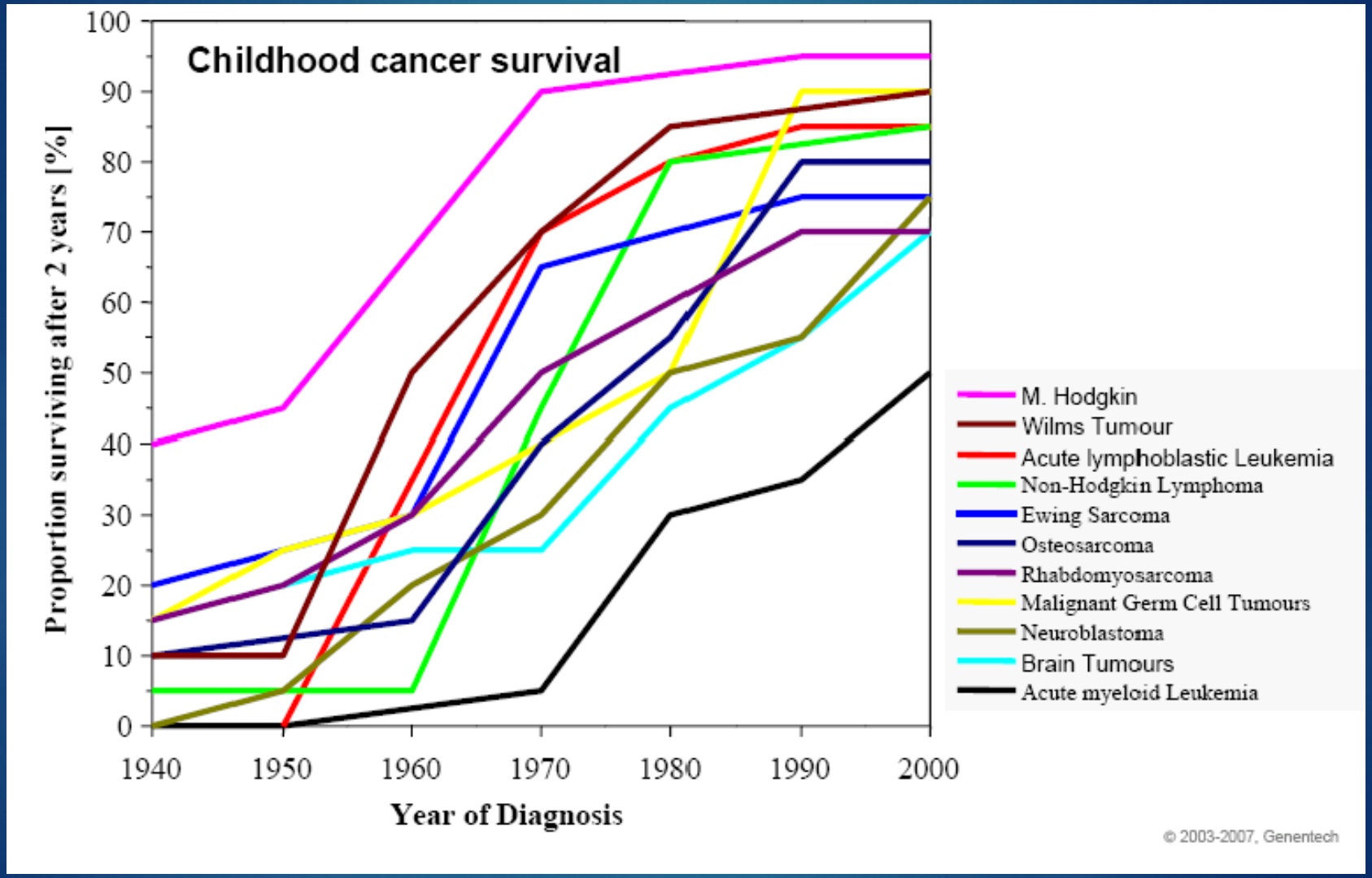
- Pediatric Cancer – PPB and OTHERS
- The Idea Behind the Project – Cancer stem cells
- The PDX model and preliminary results
- What is loss of imprinting
- LOI importance in detection, diagnosis and prognosis in cancer
- Our Project – Aim, methods, analyzing Data and initial results
- Discussion and Future Tasks

Pediatric Malignancies

12,400 children per year are expected to be diagnosed with cancer in the U.S.A



2-Year Survival Rate

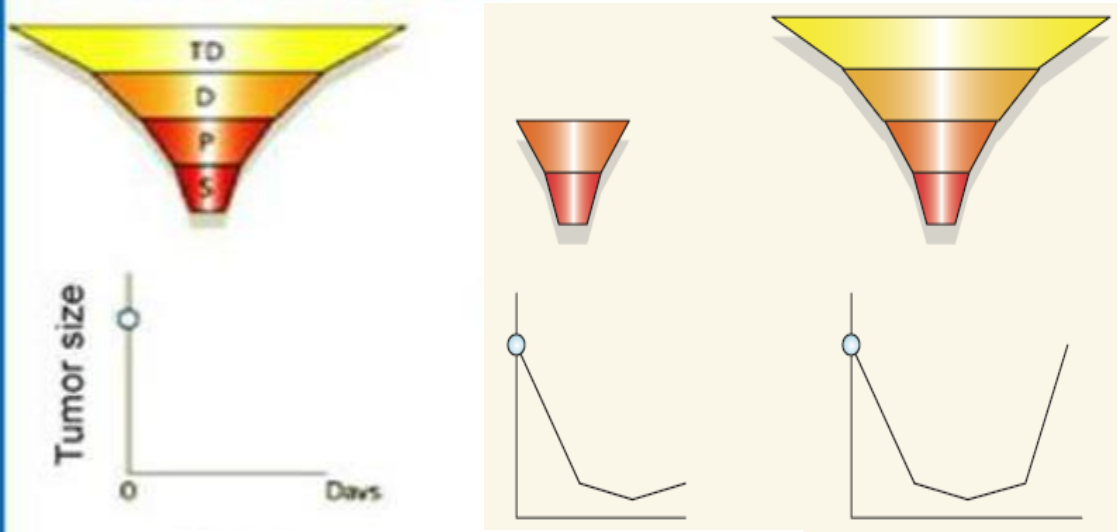


Cancer is the first-leading cause of death from disease among children

Cancer stem cell concept in tumor biology

Standard treatment does not eliminate the root of the problem

- Resistance to therapy

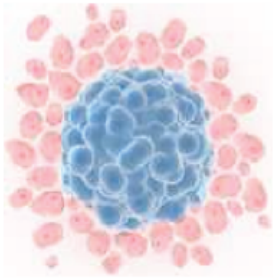


Prior to chemotherapy

Resistance or end of chemotherapy

Relapsed Disease

Modified from Huntly BJ & Billiland DG Nature 2006



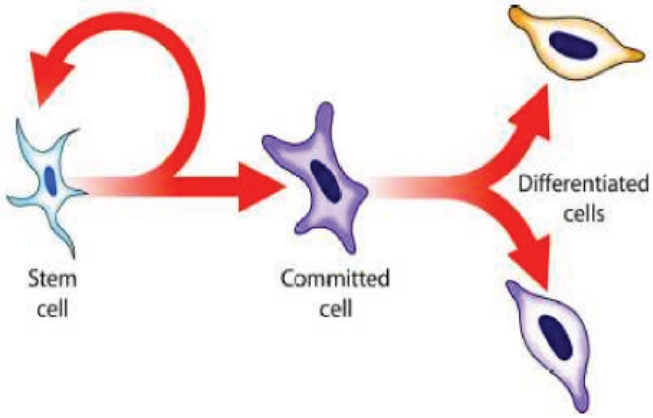
- **What is a stem cell?**

  A cell that has the ability to continuously divide and differentiate (develop) into various other kind of cells /tissues.

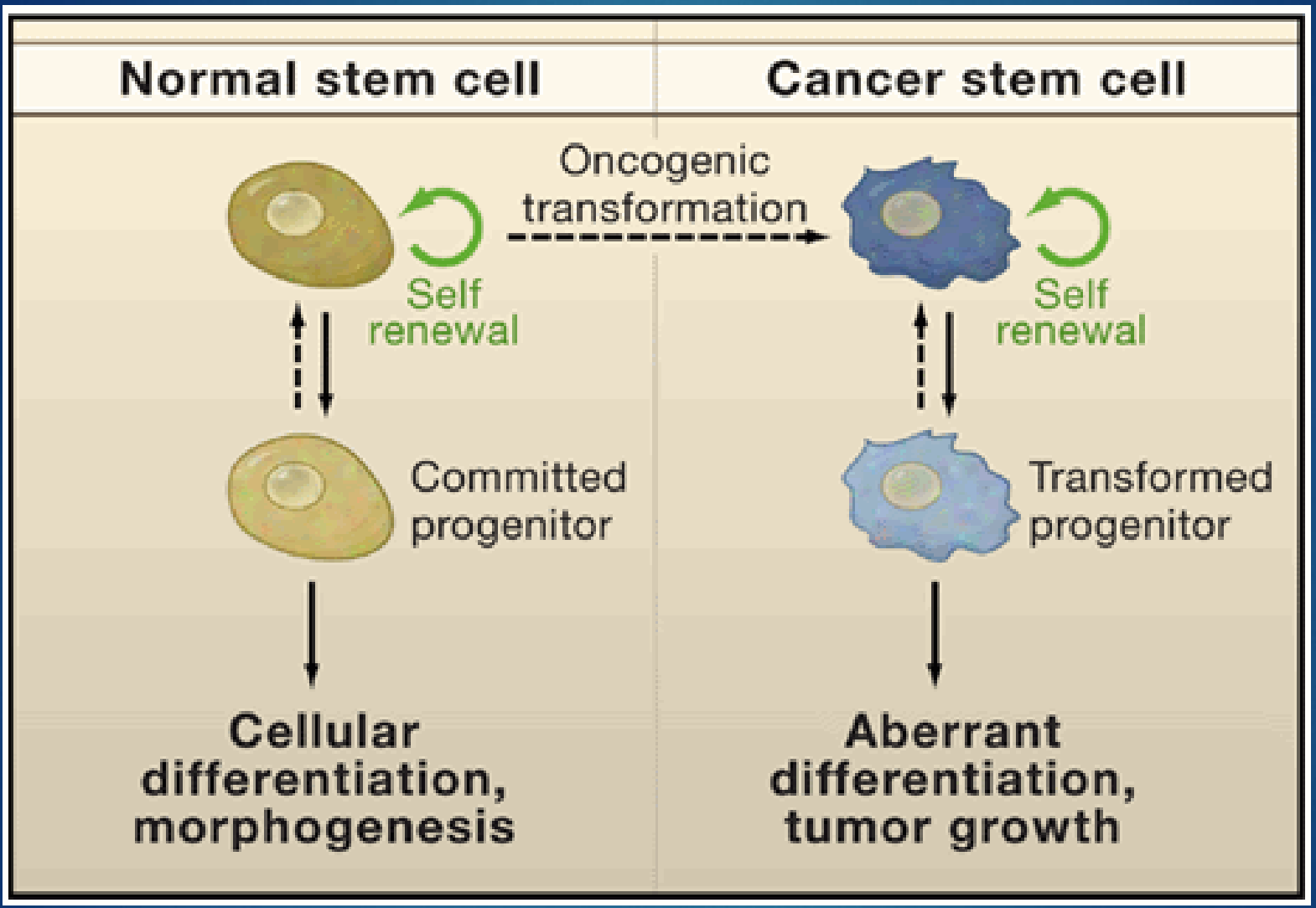
- **A stem cell has 2 key properties**

  - **Pluripotency -** the ability to differentiate into nearly all cells of the three germ layers (ectoderm, endoderm, and mesoderm)

  - **Self-renewable -** the ability of going through numerous cycles of cell division while maintaining its undifferentiated state

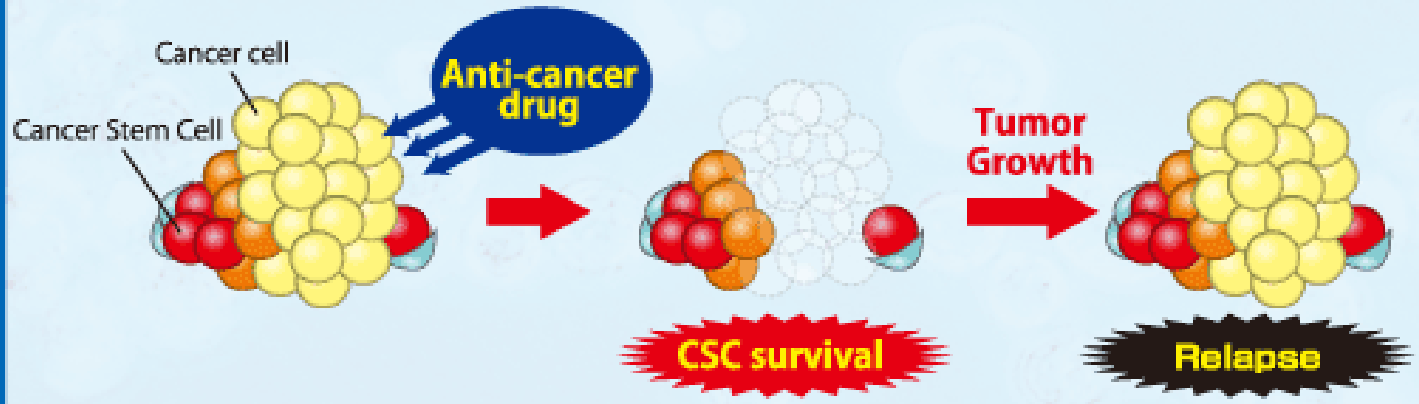


Cancer Stem Cells



Cancer Stem Cell [CSC] Characteristics

- Minor population in tumor : 0.1 - a few percent
- Self-renewing; infinite proliferative potential.
- Enhanced resistance to drugs, radiation, cell stress.
- Tumorigenic; give rise to other cell types in tumor.
- Associated with **metastasis** and **relapse.**



Metastasis and relapse are involved in more than 90% of all cancer deaths

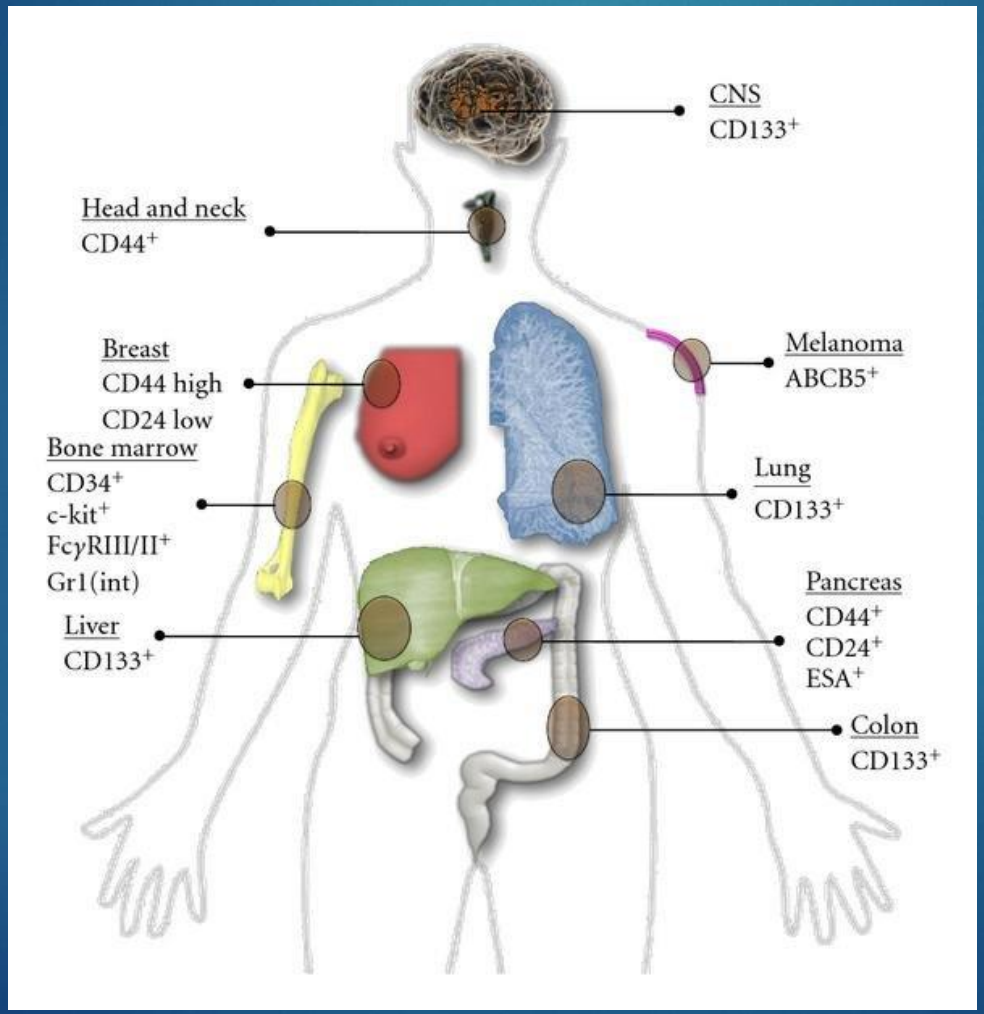
Strategies to eradicate CSCs are an urgent topic in cancer research

CSCs and cancer treatment

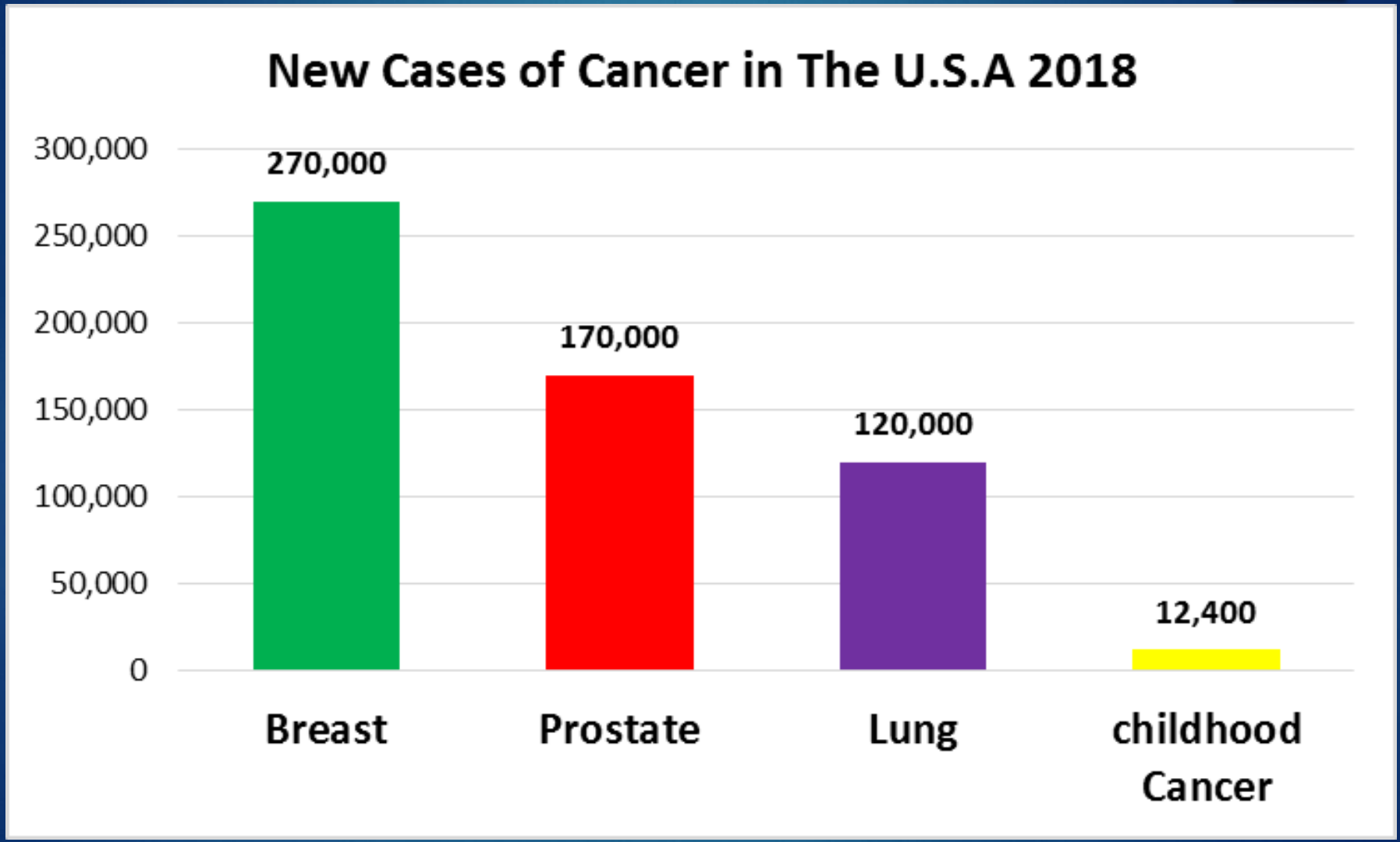
- There is a need for new interventions that reduce CSC capacity in order to prevent recurrences, extend survival, and cure many types of cancer.

- Better understanding the mechanisms of CSC resistance will help in discovery of new molecular targets for the development of better agents to eliminate or differentiate CSCs

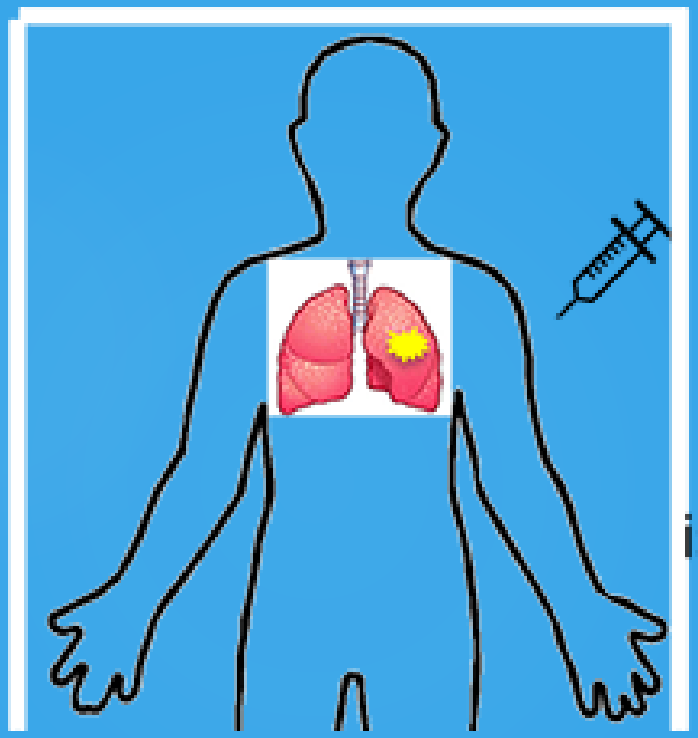
Cancer stem cell (CSC) research relies on the ability to identify and isolate these cells from fresh tumor samples



- Childhood Cancer is a rare disease…



A platform to discover therapeutic cancer stem cell antigens in rare pediatric tumors using patient derived xenografts (PDX)

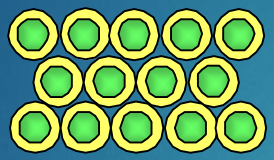


Biopsy sample of tumor
is implanted into a mouse

PDX mouse model

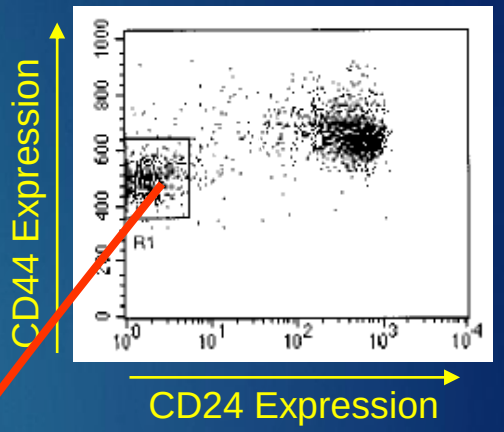
PDX model for CSC research

Solid Tumor



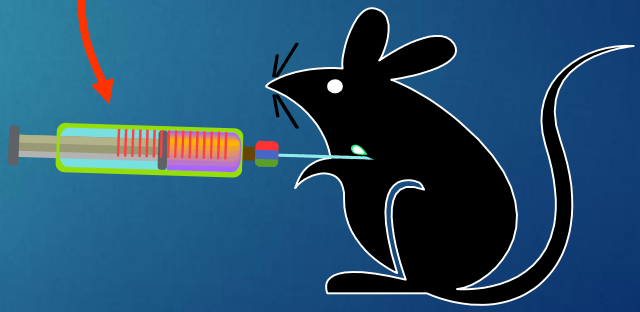
Single Cell Suspension

FACS Cell Sorter

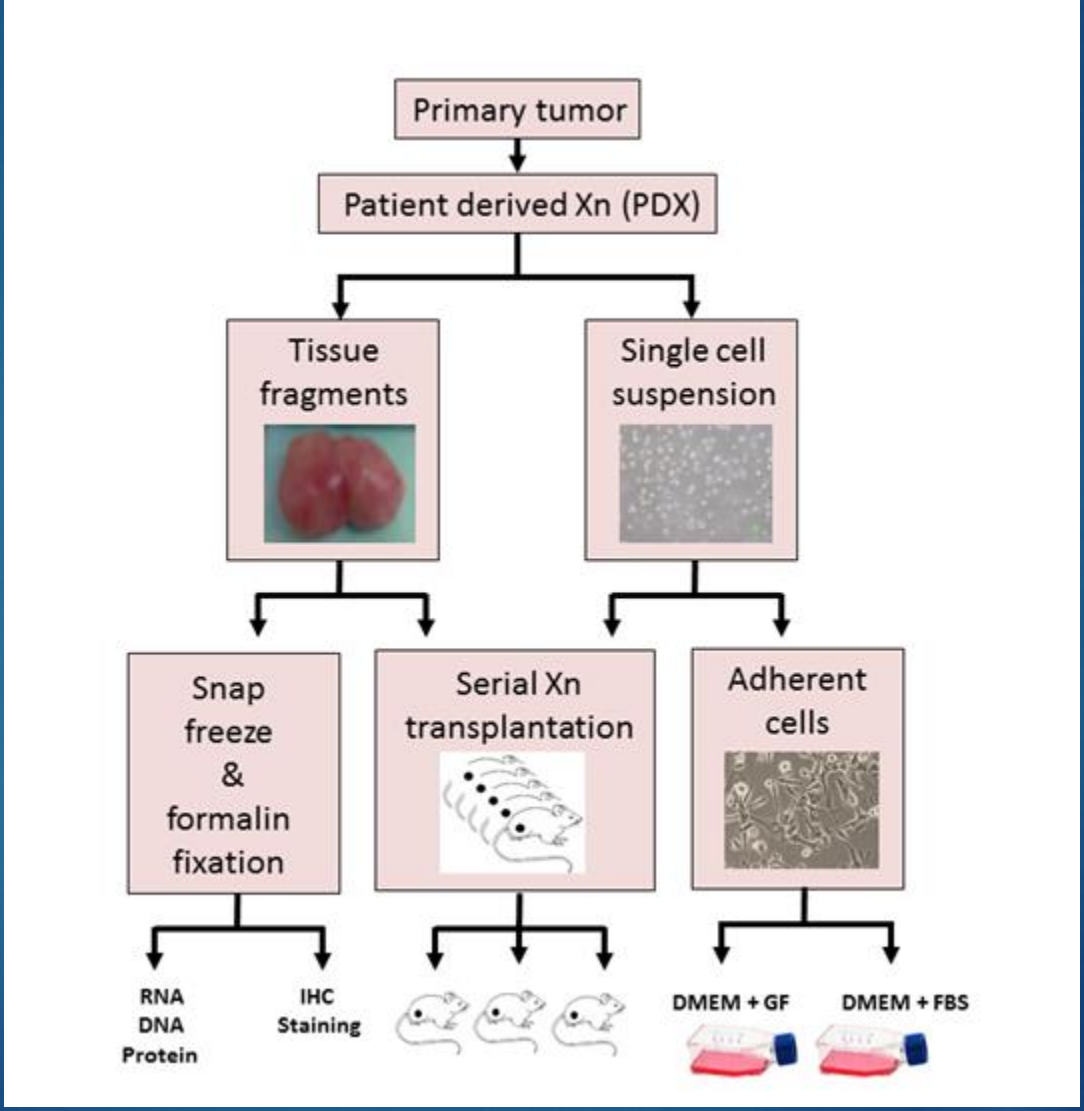


Mince (small pieces)

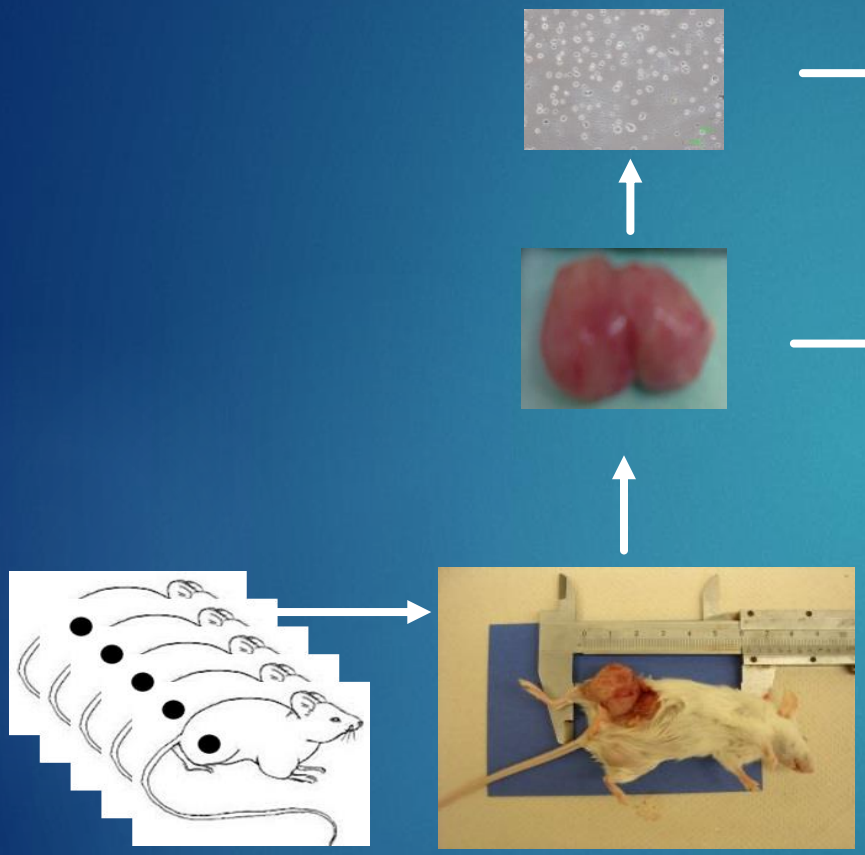
Surgical Implantation



Our Protocol for Generation and establishment of human PDX

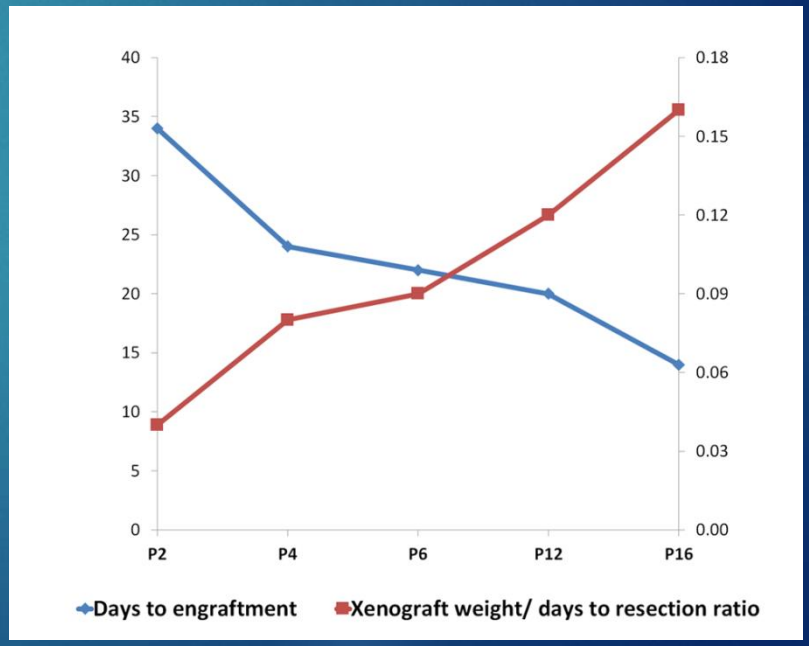


During serial propagation of PDX shorter time to tumor engraftment , accelerated tumor growth and less amount of cells needed to establish Xn were demonstrated - indicating the promotion of tumor aggressiveness and stemness along passages



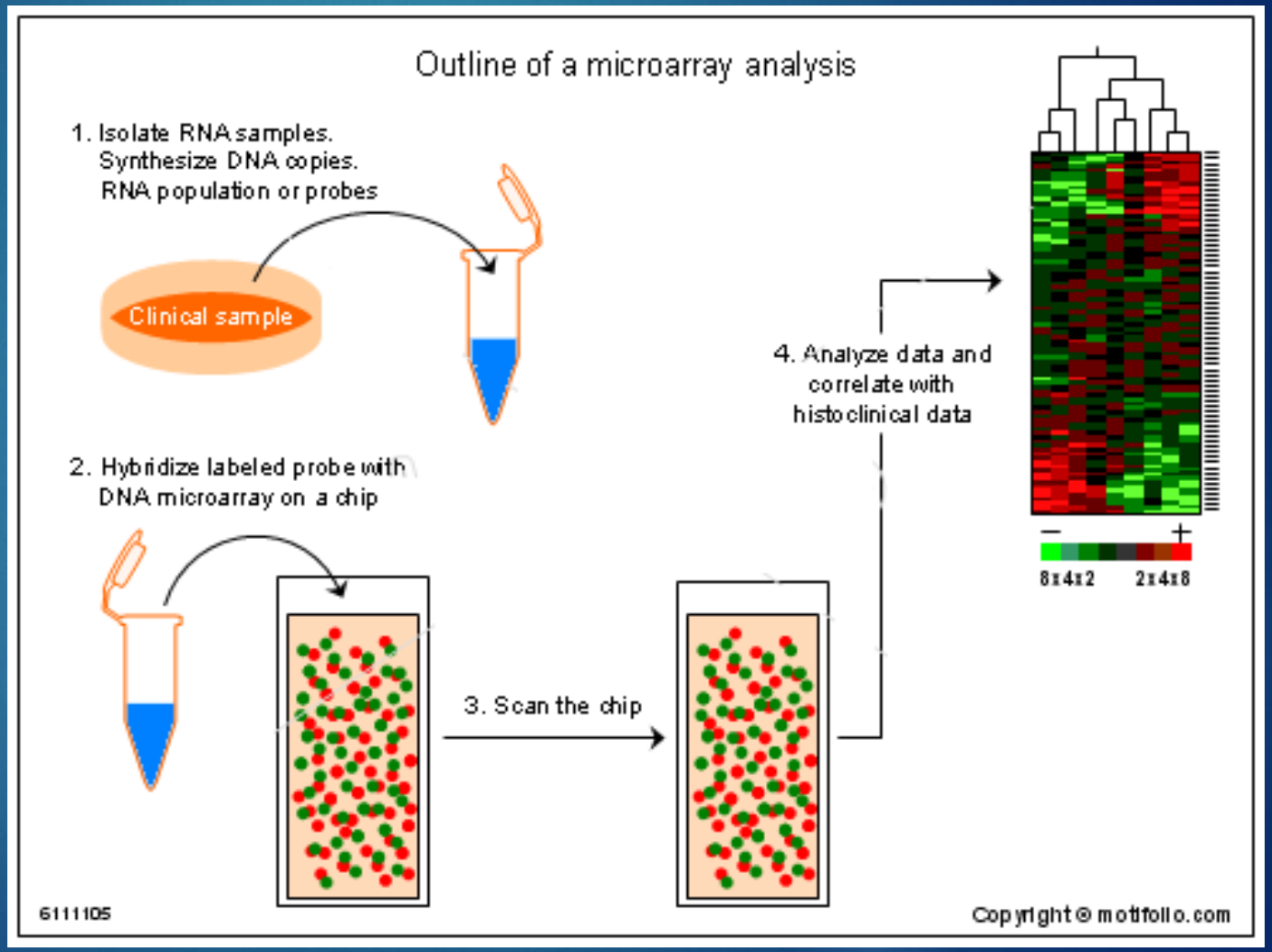
1x10⁶ cells

50 cells



Characterization of the molecular profile accompanying the selection for the CSC phenotype during Xn propagation

- Primary tumor
- Passage 2
- Passage 4
- Passage 6
- Passage 8
- Passage 12
- Fetal Tissue
- Adult Tissue



Previous Publications

- Xenograft model system of human **Wilms' tumor** that allowed to identify molecular events in WT tumorigenesis

(Dekel et al., 2006a); (Metsuyanim et al., 2008); (Pode-Shakked and Dekel, 2011)

- Identification of cancer-initiating cells that propagate and sustain WT *in vivo*

(Pode-Shakked et al., 2009); (Pode-Shakked et al., 2013); (Shukrun et al., 2014)

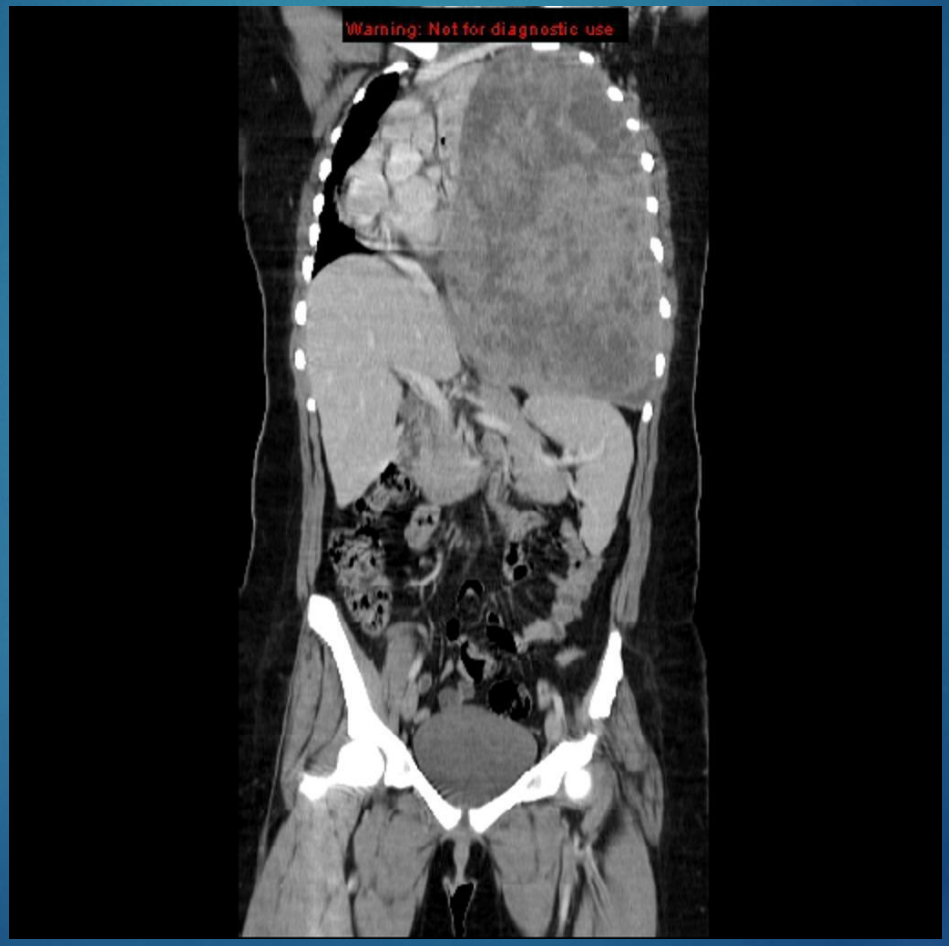
- mTORC1 Inhibition Is an Effective Treatment for Sporadic Renal **Angiomyolipoma**

(Pleniceanu O et al.,2017)

- In Vivo Expansion of Cancer Stemness Affords Novel Cancer Stem Cell Targets: **Malignant Rhabdoid Tumor** as an Example

(Golan H et al., 2018)

Pleuro Pulmonary Blastoma (PPB)



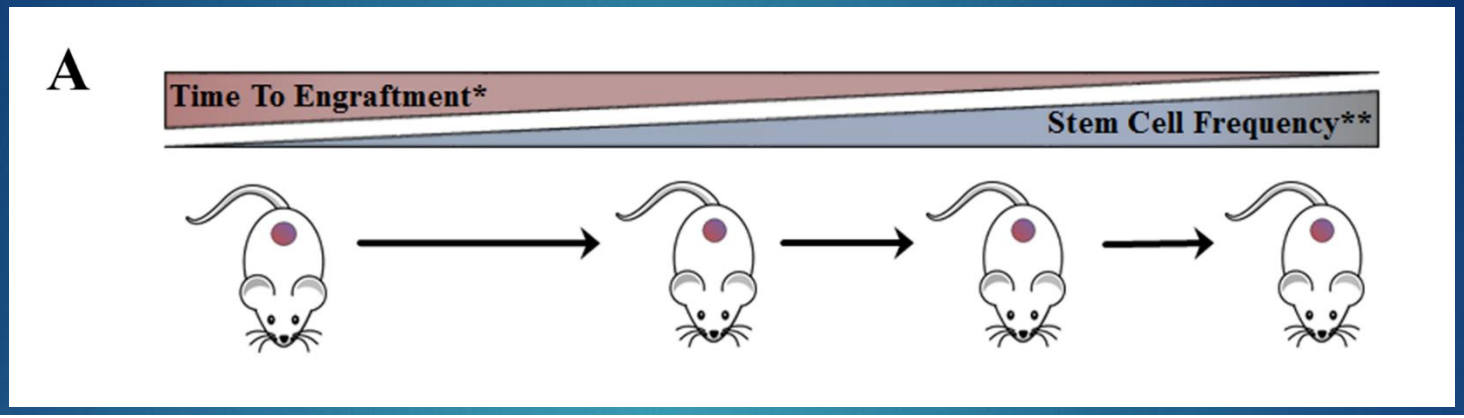
Pleuropulmonary blastoma (PPB)

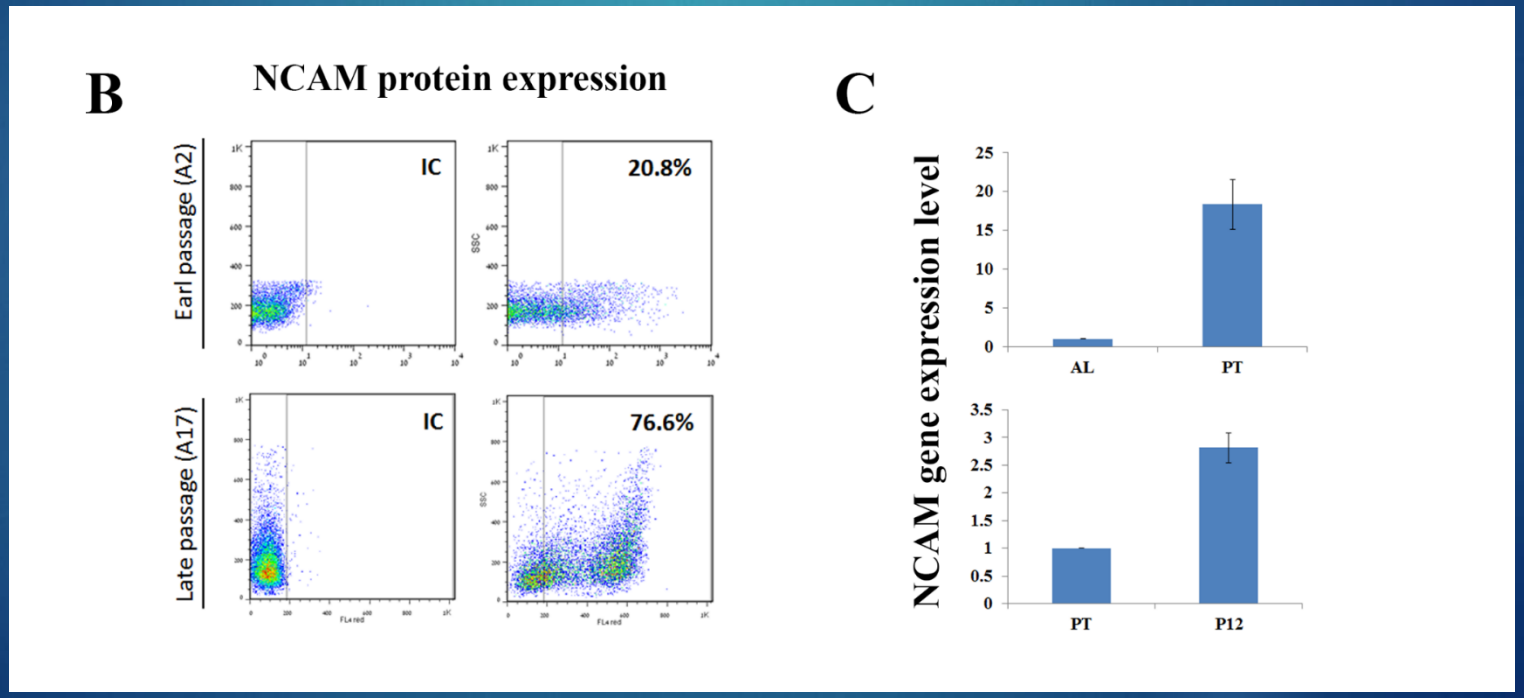
- Pleuropulmonary blastoma (PPB) is a rare, aggressive malignant tumor of intrathoracic (pulmonary, pleural, or combined) mesenchyme.
- It affects infants and children under four years of age
- PPB is categorized into three subtypes: pure cystic (Type I), solid and cystic (Type II), and pure solid (Type III).
- Germline mutation in the *DICER1* gene has been considered a major etiologic factor of this malignant sarcoma
- Types II and III are more aggressive than Type I, with poorer outcome and worse prognosis, due to earlier local recurrence and distant metastasis
- **Therefore, there is an urgent need to uncover novel therapeutic strategies**

Case Presentation

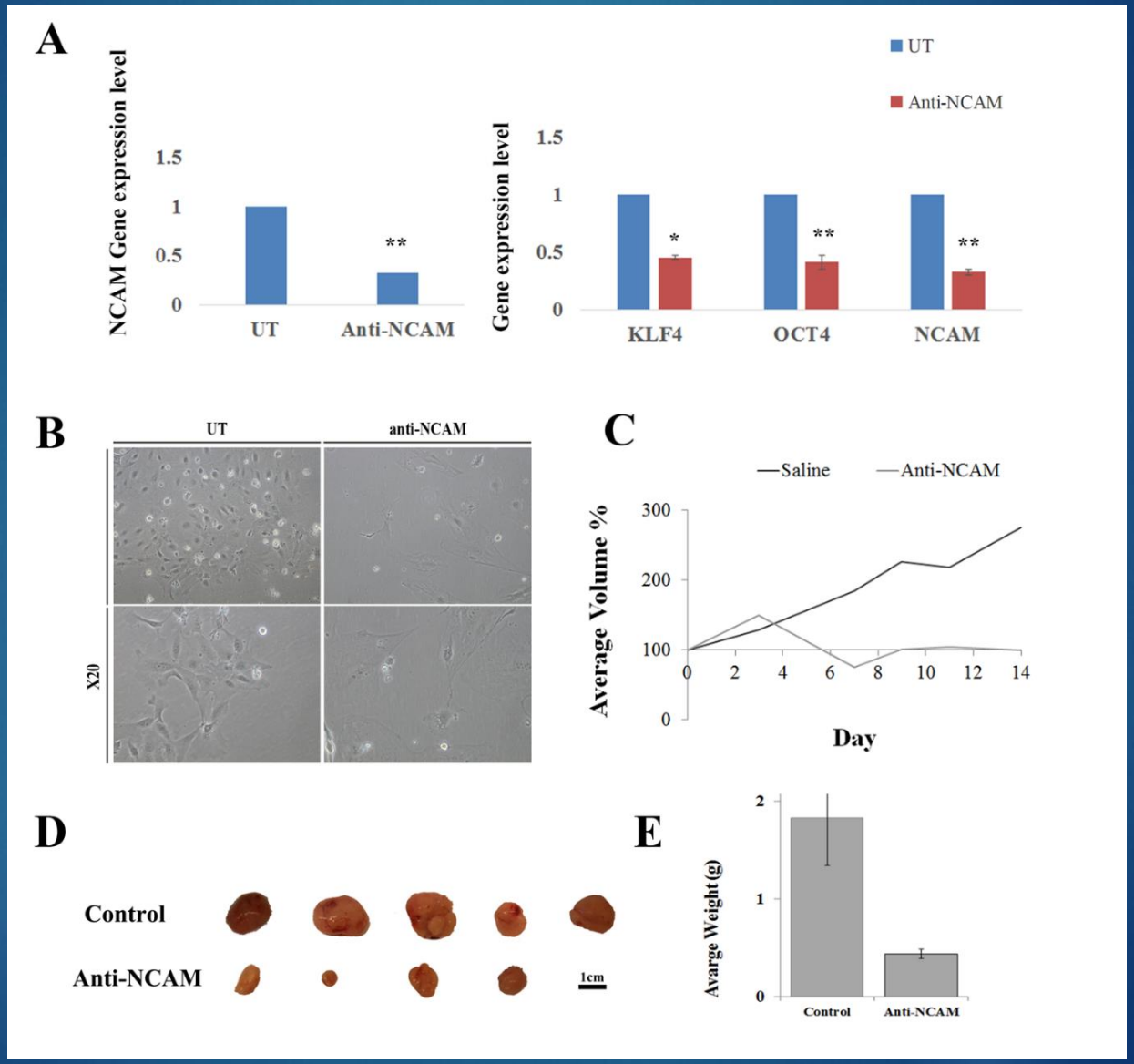
- A 3 year old male presented at 2007 with a huge Lt pleuropulmonary mass that was resected.
- He was further treated with Chemotherapy
- On 2011 a relapse was diagnosed.
- Re – operation was done followed by chemotherapy, radiotherapy and Autologous BMT
- Sample from the tumor was taken for PDX formation.

NCAM is a PPB CSC biomarker





NCAM as a PPB therapeutic target



Multiple Imprinted and Stemness Genes Provide a Link between Normal and Tumor Progenitor Cells of the Developing Human Kidney

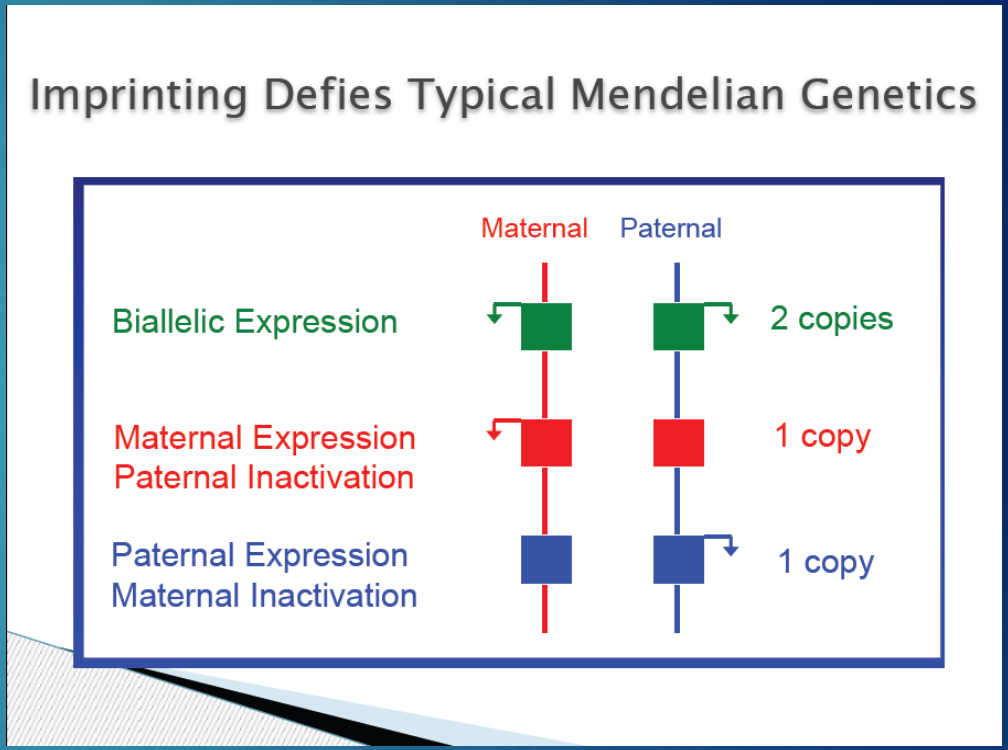
(Dekel et al., 2007)

Imprinted genes

- **Monoallelic expression**
- Involved in growth regulation and development

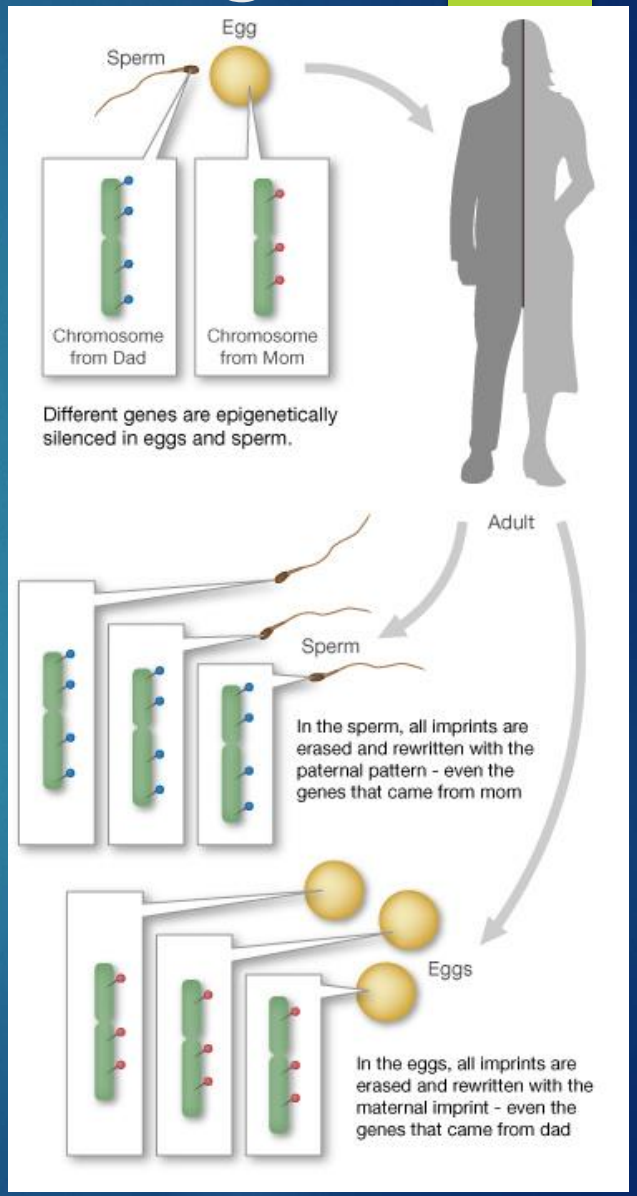
- Small subset of group that one copy is turned off in a parent-of-origin dependent manner ,**the gene was epigenetically marked or imprinted in either the egg or the sperm**

Imprinting status varies between tissues, developmental stages and species



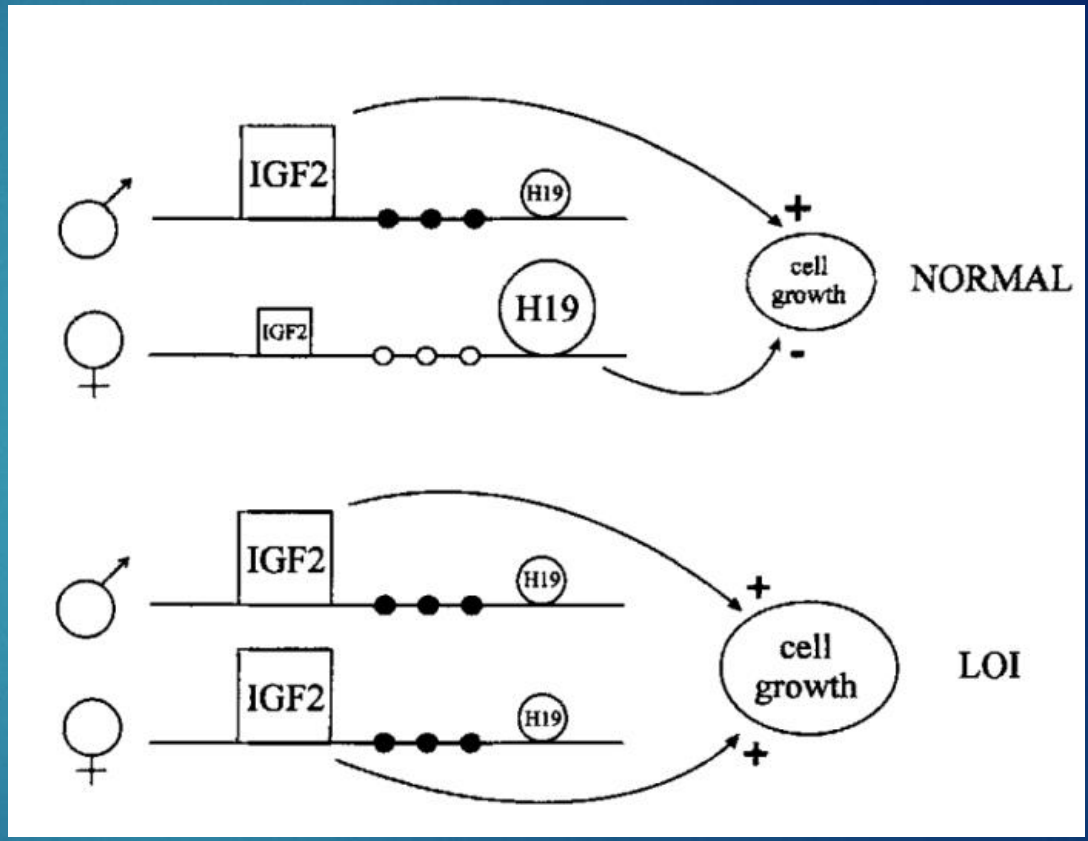
What Is Genomic imprinting?

- Expression depends upon whether it resided in a male or female the previous generation.
- In primordial germ cells imprinting erased and re-establish **according to the sex of the individual.**
- Regulation **governed by imprinted differentially methylated regions** (iDMRs),



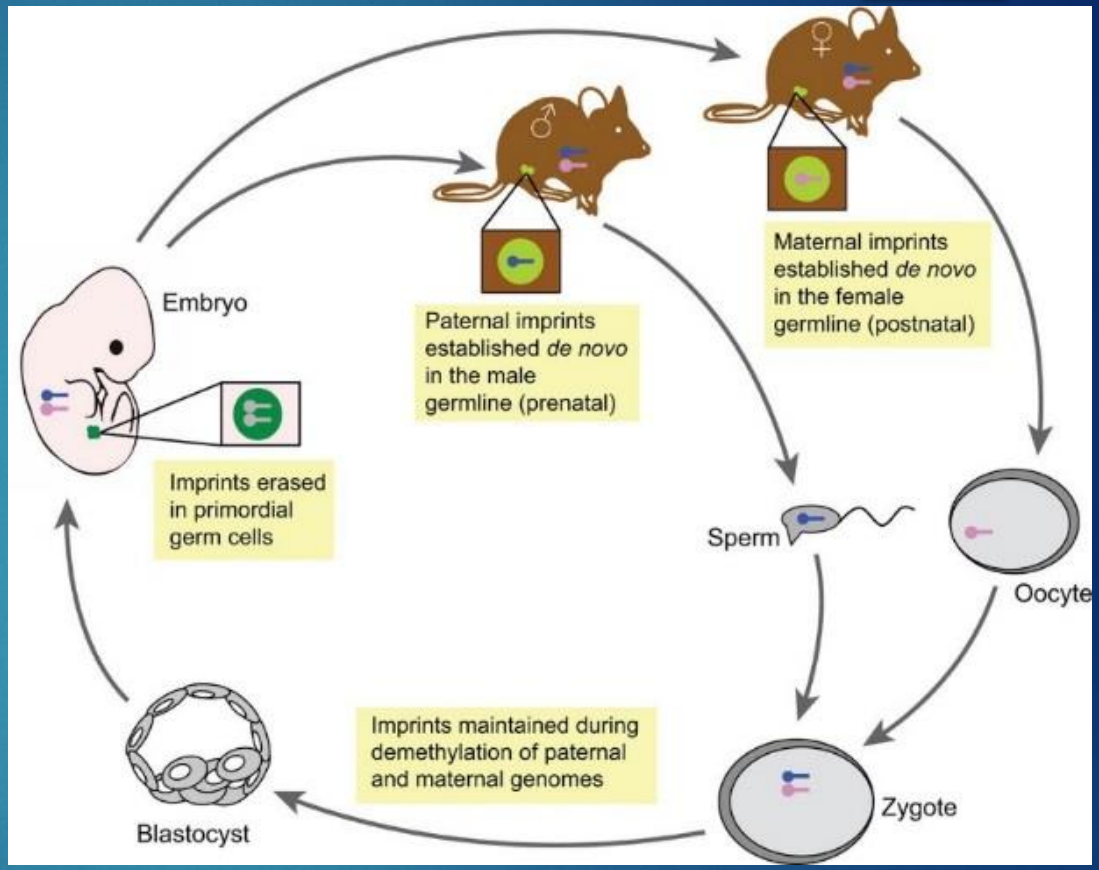
Loss of imprinting

- Loss of imprinting (LOI) is defined by the **disruption of the normal monoallelic expression pattern** of an imprinted gene.
- LOI may result in either **complete (biallelic) silencing or biallelic transcription** that is thought to cause higher expression levels, mainly by alterations in DNA methylation at iDMRs.

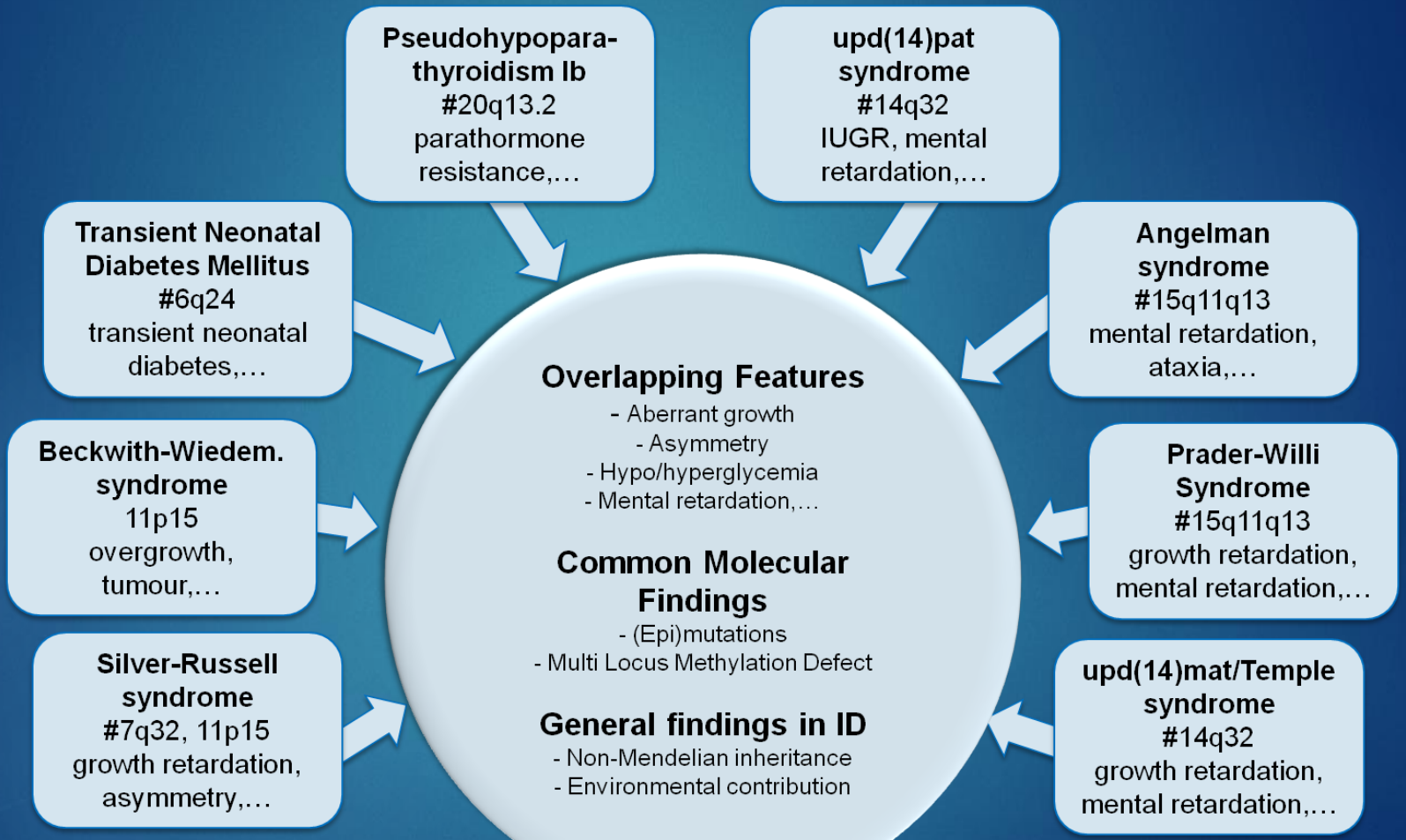


Role Of LOI in Abnormaliries

- **MAT** expressed genes: Growth suppressors (TSGs?)
- **PAT** expressed genes: Growth promoters (Oncogenes?)



Imprinting Disorders



LOI in cancer

Cancer type	Incidence of LOI (% of cases)
Chronic myeloid leukemia	100
Ovarian tumors	80
Wilms' tumors	70
Colorectal cancer	66
Barrett's eosphagus	56
Renal-call carcinomas	50
Eosphageal cancer	50
Meningiomas	30
Lung adenocarcino-osteosarcoma	47–85
Others*	variable

The idea behind the project

Background

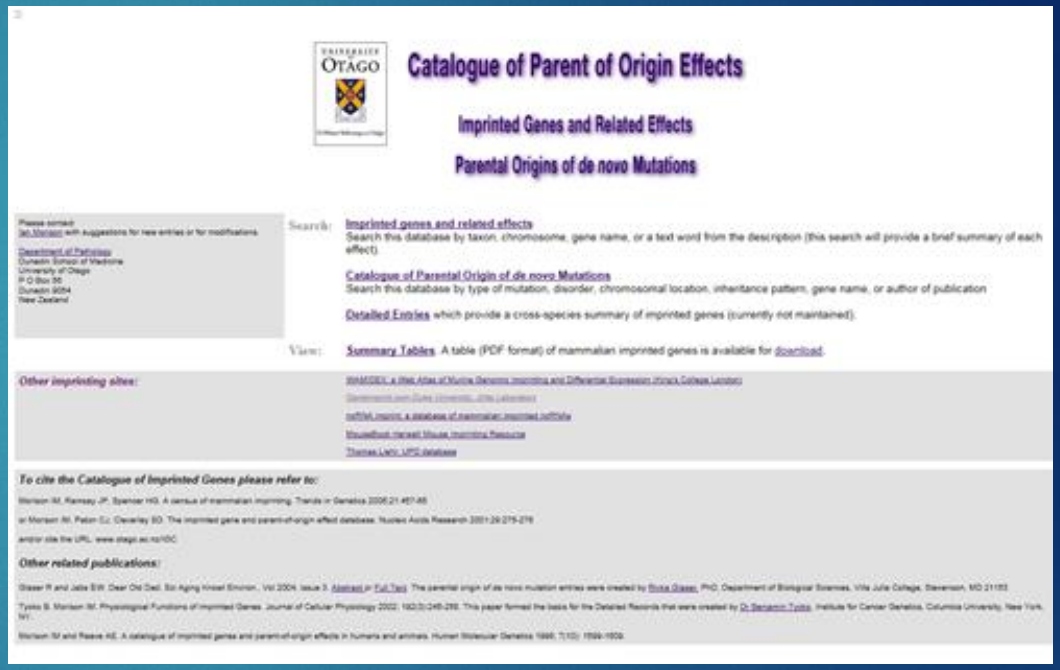
- The importance of Loss of imprinting on pediatric tumors and its role in stem cells
- Understanding different processes before the tumor reveals
- Learning more about PPB specifically
- Epigenetics may be reversible? Intervention as Therapy?

Aim

- **To identify changes in the pattern of imprinted genes expression along tumor progression in XN models of pediatric tumors**

Back to Research Methods and analyzing

- **Identify "imprinting genes"** from medical sources and other projects, such as: http://igc.otago.ac.nz/home.html
- **Classifying** imprinting genes to 3 categories:
  - Single isoform - monoallelically expressed in **all** normal tissues.
  - tissue- or **isoform-specific** imprinted genes
  - **provisional or conflicting** evidence for imprinting



List of Imprinted genes

Table S2 - List of analyzed imprinted genes (Related to Figure 1)							
Gene	Region	Chr	Start	Stop	Size (bp)	Imprinting Status	Expressed allele
DIRAS3	1p31	chr1	68511644	68516481	4,837	Single Isoform	Paternal
LRRTM1	2p12	chr2	80515480	80531487	16,007	Single Isoform	Paternal
ZDBF2	2q33.3	chr2	207139522	207179148	39,626	Single Isoform	Paternal
GPR1-AS	2q33.3	chr2	207068100	207130967	62,867	Single Isoform	Paternal
NAP1L5	4q22.1	chr4	89617065	89619023	1,958	Single Isoform	Paternal
HYMAI	6q24.2	chr6	144324033	144329867	5,834	Single Isoform	Paternal
FAM50B	6p25.2	chr6	3850045	3851551	1,506	Single Isoform	Paternal
SGCE	7q21.3	chr7	94214535	94285521	70,986	Single Isoform	Paternal
PEG10	7q21.3	chr7	94285636	94299006	13,370	Single Isoform	Paternal
MESTIT1	7q32.2	chr7	130126897	130131013	4,116	Single Isoform	Paternal
KLF14	7q32.3	chr7	130417395	130418888	1,493	Single Isoform	Maternal
H19	11p15.5	chr11	2016405	2019065	2,660	Single Isoform	Maternal
IGF2	11p15.5	chr11	2150341	2170833	20,492	All isoforms are imprinted	Paternal
IGF2AS	11p15.5	chr11	2161750	2169903	8,153	Single Isoform	Paternal
INS	11p15.5	chr11	2181008	2182439	1,431	Single Isoform	Paternal
KCNQ1OT1	11p15.5	chr11	2629557	2655367	25,810	Single Isoform	Paternal
KCNQ1DN	11p15.5	chr11	2892666	2893336	670	Single Isoform	Maternal
RTL1	14q32.2	chr14	101346991	101351184	4,193	Single Isoform	Paternal
DLK1	14q32.2	chr14	101193201	101201467	8,266	Single Isoform	Paternal
MEG3	14q32.2	chr14	101292444	101327360	34,916	Single Isoform	Maternal
MEG8	14q32.2	chr14	101361106	101373305	12,199	Single Isoform	Paternal
MKRN3	15q11-q13	chr15	23810453	23856711	46,258	Single Isoform	Paternal
MAGEL2	15q11-q13	chr15	23888695	23892993	4,298	Single Isoform	Paternal
NDN	15q11-q13	chr15	23930553	23932450	1,897	Single Isoform	Paternal
NPAP1	15q11-q13	chr15	24920540	24928593	8,053	Single Isoform	Paternal
SNRPN	15q11-q13	chr15	25068794	25200068	131,274	All isoforms are imprinted	Paternal
NAA60 (Monoallelic isoform)	16p13.3	chr16	3493668	3507990	14,322	Imprinted Isoform Only	Maternal
ZNF597	16p13.3	chr16	3482422	3493537	11,115	Single Isoform	Maternal
MIMT1	19q13.43	chr19	57352269	57359922	7,653	Single Isoform	Paternal
PEG3 (Monoallelic isoform)	19q13.43	chr19	57336372	57352097	15,725	Imprinted Isoform Only	Paternal
PSIMCT-1	20q11.21	chr20	30135185	30136019	834	Single Isoform	Paternal
SGK2 (V1)	20q13.12	chr20	42187635	42214273	26,638	Single Isoform	Paternal
SGK2 (V2)	20q13.12	chr20	42168791	42198156	29,365	Single Isoform	Paternal
GNAS-AS1 (non-overlapping region)	20q13.2	chr20	57393951	57425984	32,033	Imprinted Isoform Only	Paternal
NNAT	20q11.2	chr20	36149606	36152090	2,484	Single Isoform	Paternal

Methods and Analyzing

- **Samples**: tumor tissue ,adult healthy tissue and the fetal healthy tissue, transfer in Xenograft.
- Significant difference of gene expression:

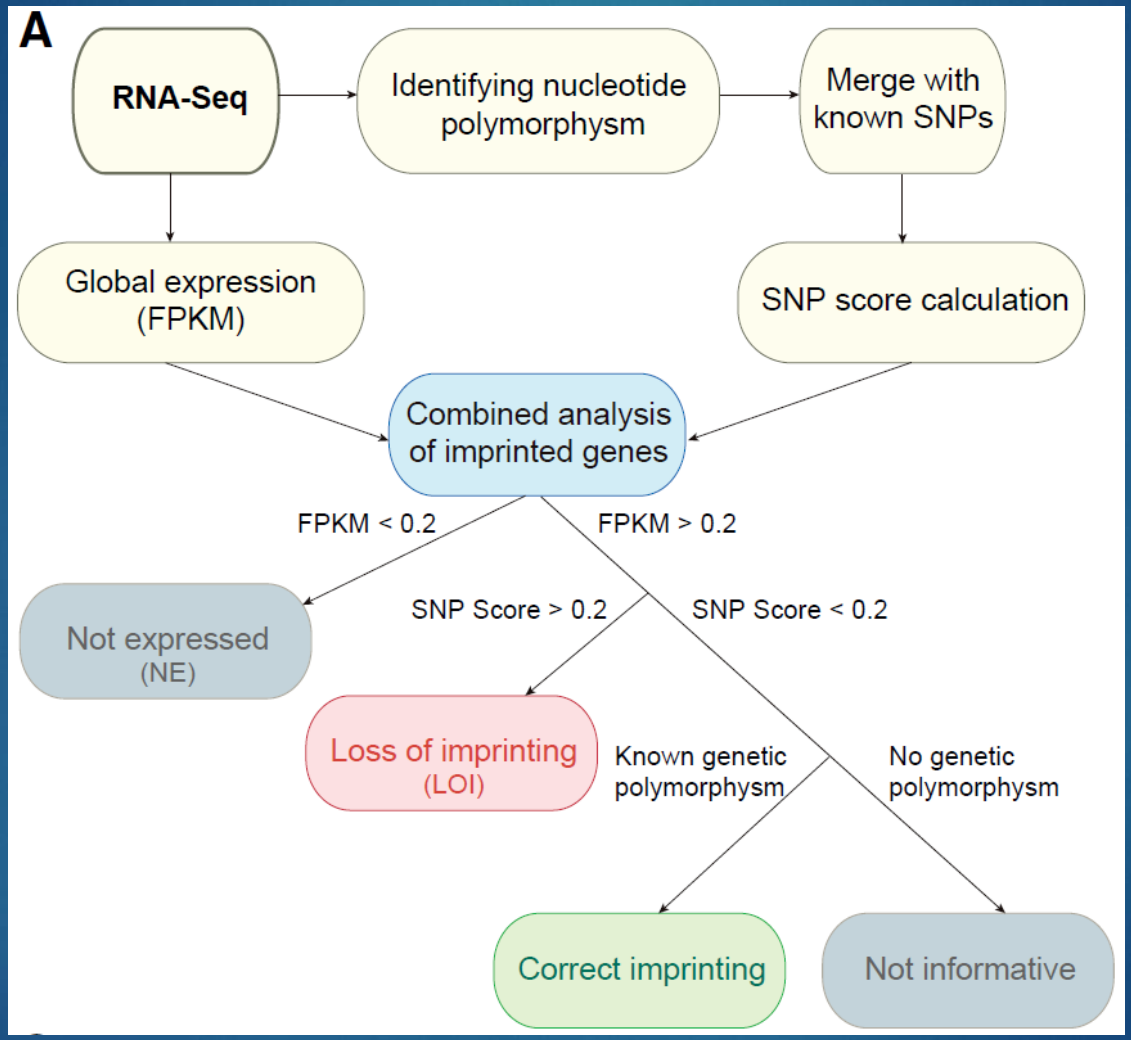
"fold change" >2 or fold change<0.5

- **population** :
  - microarray expression Of PPB
  - Comparison to RNA-seq of 4 pediatric tumors that were progressed in XN models including : MRT, EWING sarcoma, Wilms and medulloblastoma.

Comparison between different tissues – Part of the tumor process or the transfer process?

Tissue A vs. Tissue B	Possible conclusion
Primary tumor vs. Adult lung	Identify tumorigenic genes
Primary tumor vs. Fetal lung	Developmental genes
Fetal lung vs. Adult lung	Genes of development and growth
tumor vs. healthy lung	Changes are connected to proliferation of the tumor
Primary tumor vs. P12 (transfer)	Does the tumor become more aggressive between the transfer/ cancer stem cells?

Collaboration In Bioinformatics



Discussion and Future

- Broad phenomenon or specific cancer (blastemal)?
- Identifying genes that can serve as prognostic markers and help stratifying patients into risk groups
- Identifying genes role in biological processes and pathways for personalized targeted treatments

Future Treatments