
Targeting cancer stem cells: strategies
to identify novel oncogenes and chemotherapeutics
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Cancer Stem Cells and Neuroblastoma: do they exist, and if so, are they important?

Cancer Stem Cell Hypothesis

Malignancies are driven by rare cancer "stem cells" that are inherently resistant to current therapeutics

Why is this model attractive?

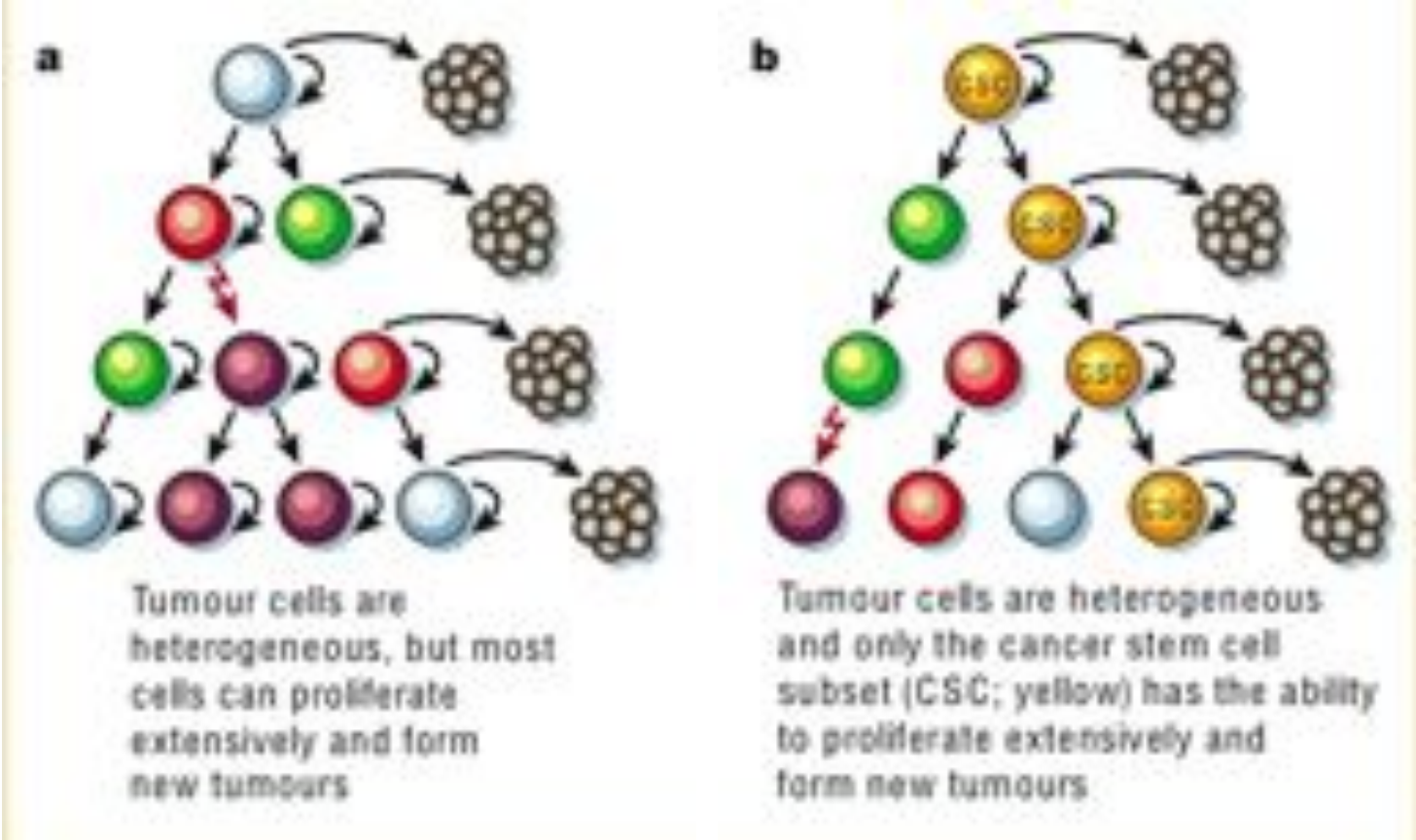
Model addresses:

Why cells in tumors are often heterogenous, and why it typically takes 1 million cells from a human tumor to form a tumor in mice

Neuroblastoma, as a heterogenous tumor that is thought to arise from a progenitor/ stem cell, is an ideal candidate for a CSC-driven tumor

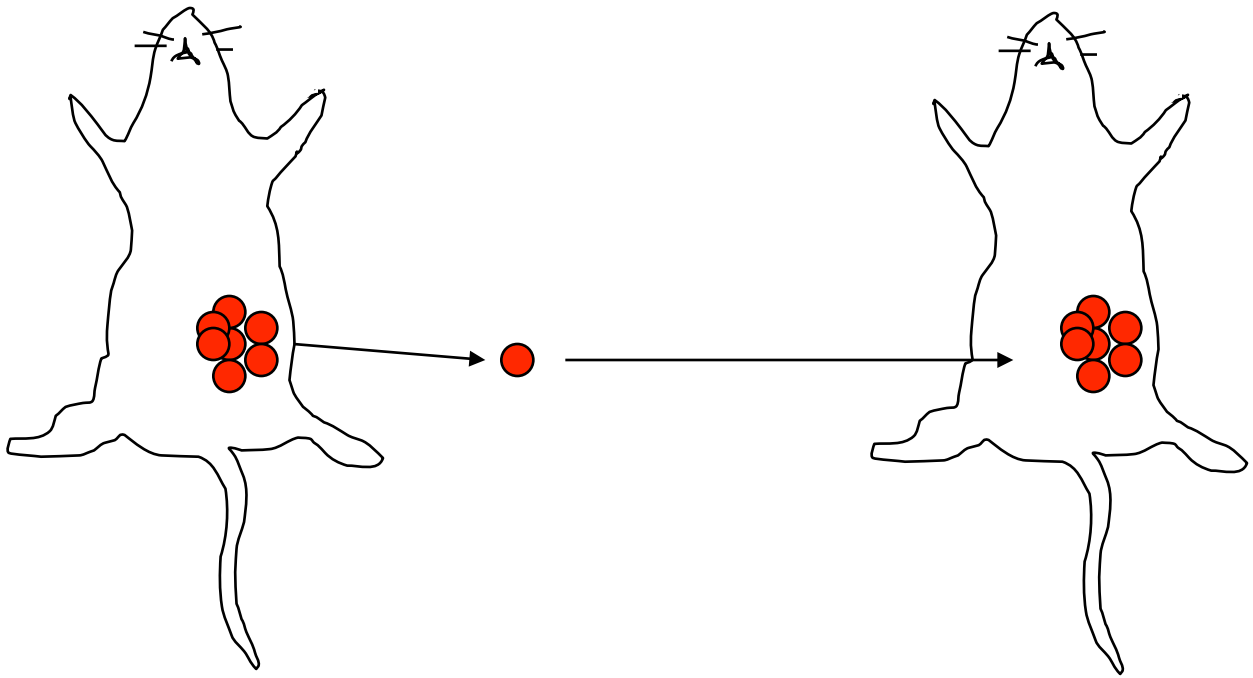
Two models of tumor development

Stochastic model Hierarchical model

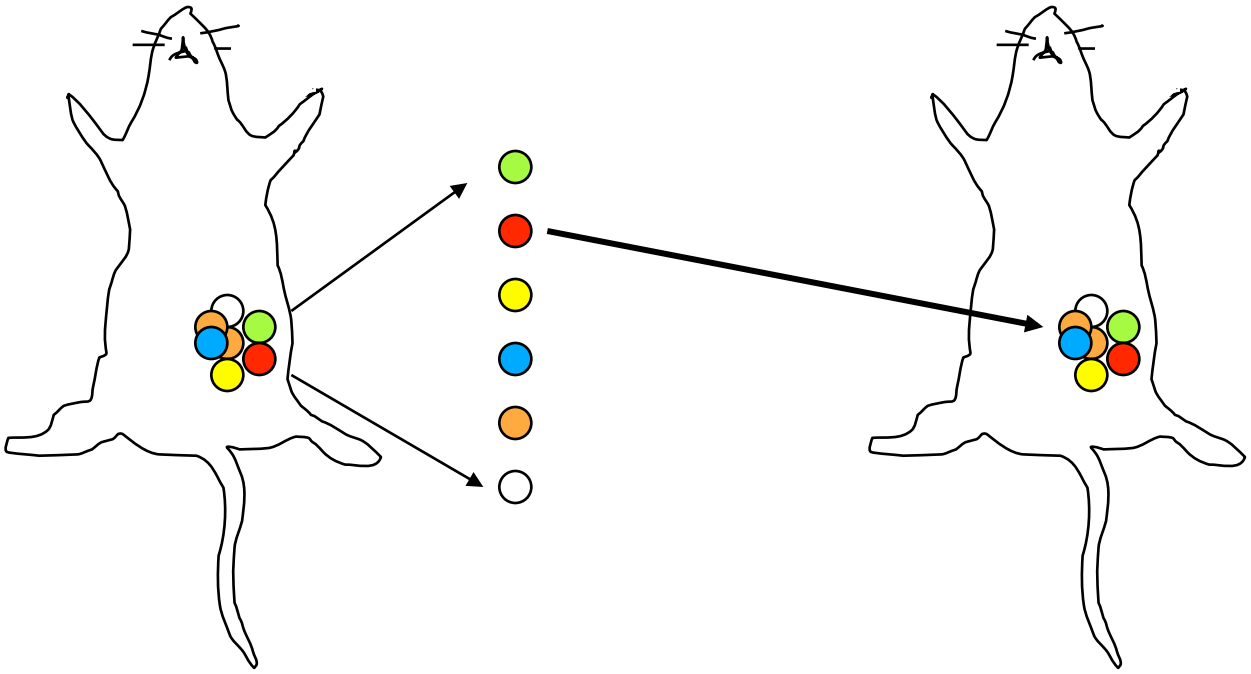


Nature 414:105, 2001

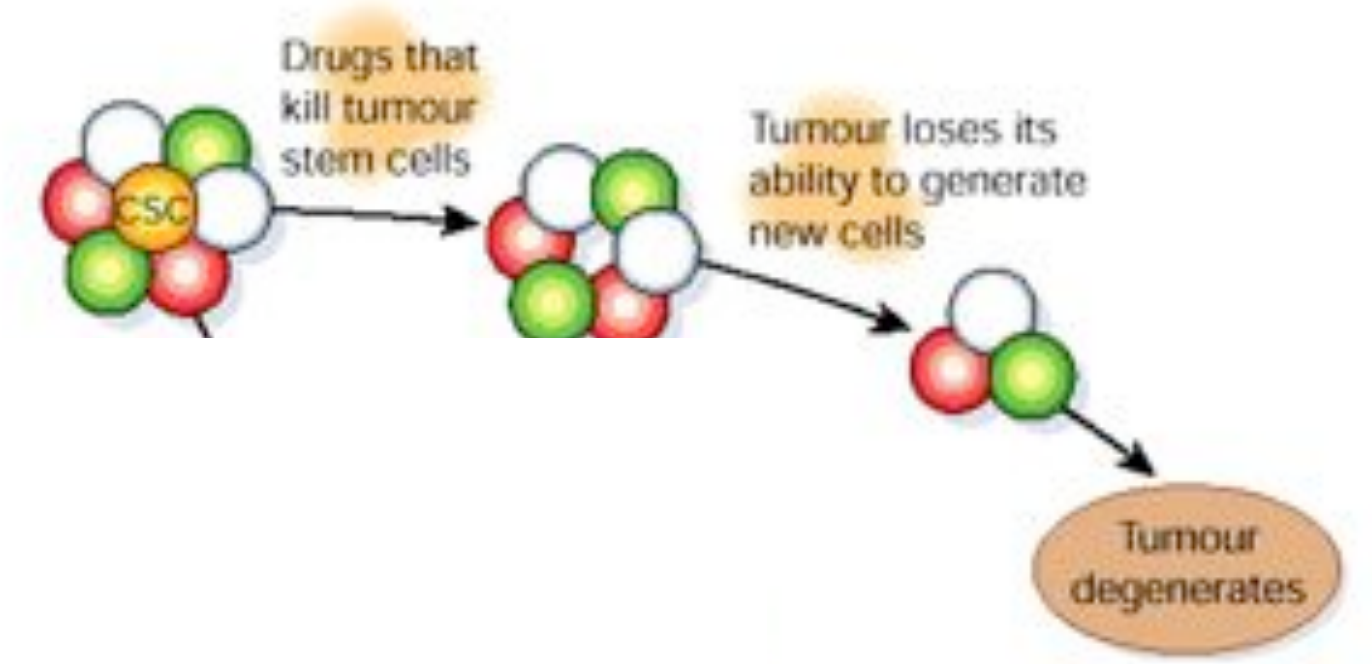
Non-stem tumor model:
every cell in a tumor should initiate a new tumor



Cancer stem cell model: only rare cells in a tumor can transplant a new tumor



Therapeutic implications of Cancer Stem Cells



Hypothesis:
Most therapies (chemotherapy and radiation) target rapidly proliferating, non-tumorigenic cells and spare the relatively quiescent cancer stem cells

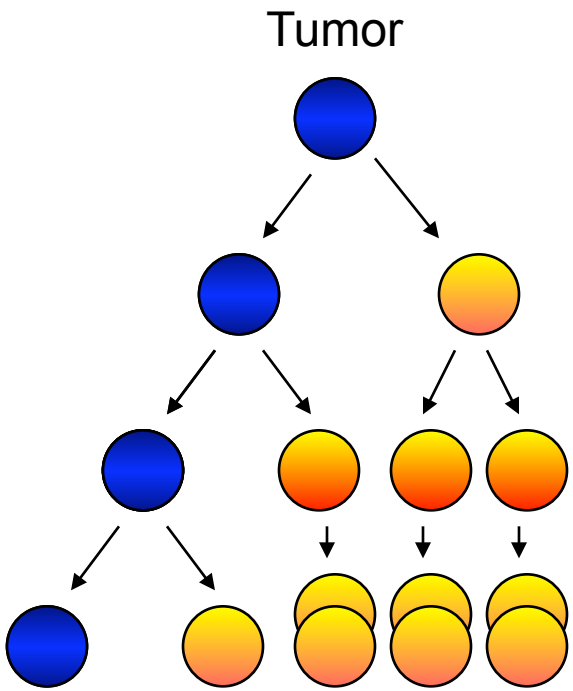
Cancer stem cells are like "queen bees." If you don't kill the queen, the hive will persist

THE JAMES FUND
For Neuroblastoma Research at Sick Kids

What is a Cancer Stem Cell?

- express stem cell markers
- self-renewal potential in culture and in vivo
- differentiation potential
- karyotypic abnormalities
- recapitulate disease *in vivo*
- hierarchy of tumor potential - important!

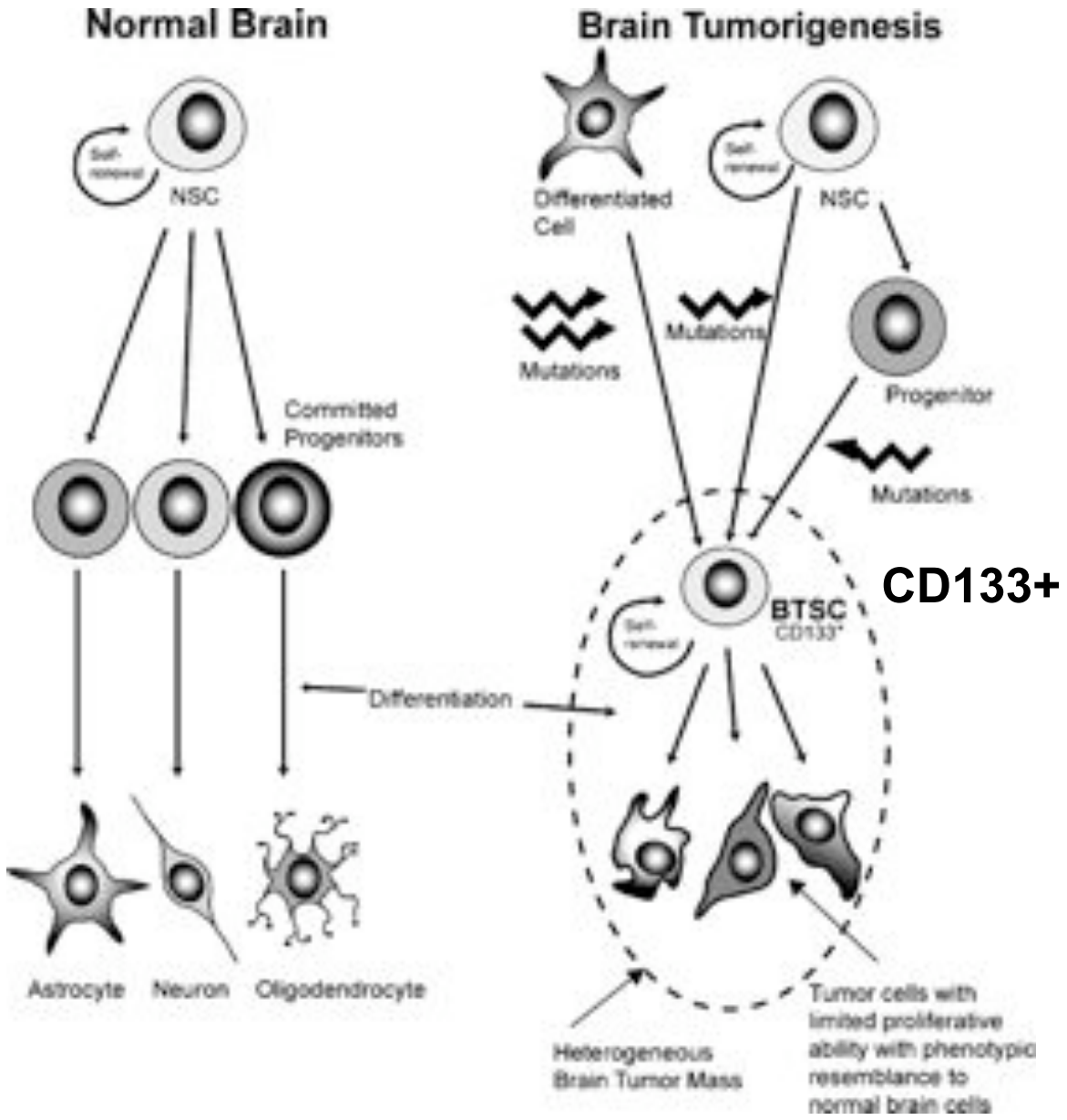
Hierarchy of CSC tumor growth



Tumor stem cell = tumorigenic

Non-tumorigenic cell

Cancer stem cells in brain tumors



Peter Dirks

Problems with the cancer stem cell hypothesis

1. Numbers! Best enrichment for CSCs is 1 in 20 (CD44+/CD24low/ALDH+ breast cancer cells), but more typically 1 in 100 (leukemia, brain, colon)

2. Available markers are poor: CD133+ cells are weakly tumorigenic - frequency of 1 in 3 in brain tumors, yet only 1 in 100 CD133+ cells form tumors

Why? Xenograft model issue: tumorigenicity is assessed in non-histocompatible host in a non-autologous niche

3. Some tumors have very high tumor-initiating frequency (neuroblastoma)

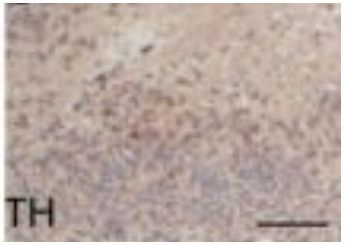
Tumor-initiating cells for neuroblastoma

- Most fatal extra-cranial malignancy of children
- Tumor of neural crest progenitors
- Few identified oncogenes or tumor suppressors (N-myc, Alk, TrkA)
- Patients often relapse multiple times after chemotherapy with bone marrow disease
- Less than 30% survival in metastatic disease

Question: Is the reason why NB often reoccurs due to a cancer stem cell that is resistant to chemotherapy?

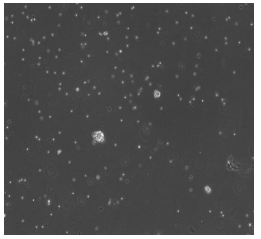
Approach: Use bone marrow metastases as well as tumors as potential sources of cancer stem/tumor-initiating cells

Identifying NB TICs



tumor

bone marrow metastasis



Mince sample

40 µm filter sample

Enzymatic digestion and/or mechanical trituration

Spheres expand in SKPs media - B27 (insulin/transferrin/putracine/ selenium), FGF and EGF

Self-renewal

SNPs

Immunophenotyping

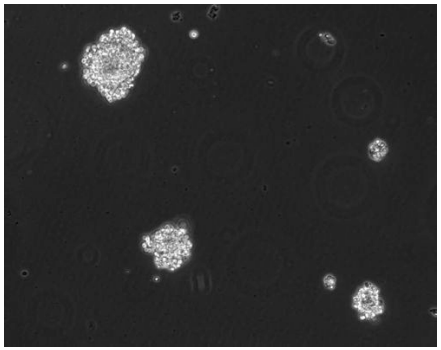
Tumorigenicity

2 weeks to 2 months to obtain TIC lines

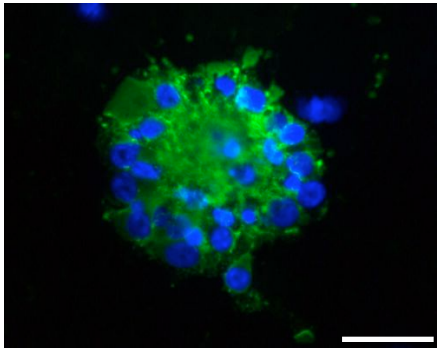
NB TIC sphere lines

- Obtained from all NB risk groups, primarily from bone marrow metastases
- Have chromosomal alterations typical of NB (Hansford et al, Cancer Research 2007)

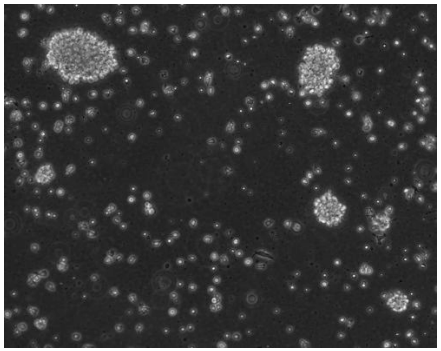
Stage 4 NB (high-risk) needle biopsy



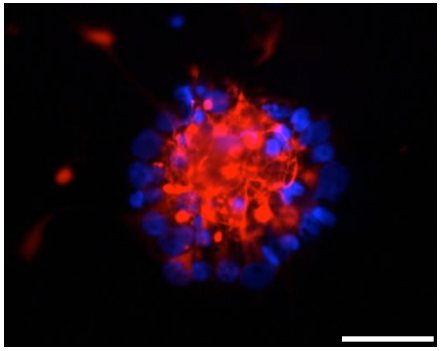
Tyrosine Hydroxylase (NB marker)



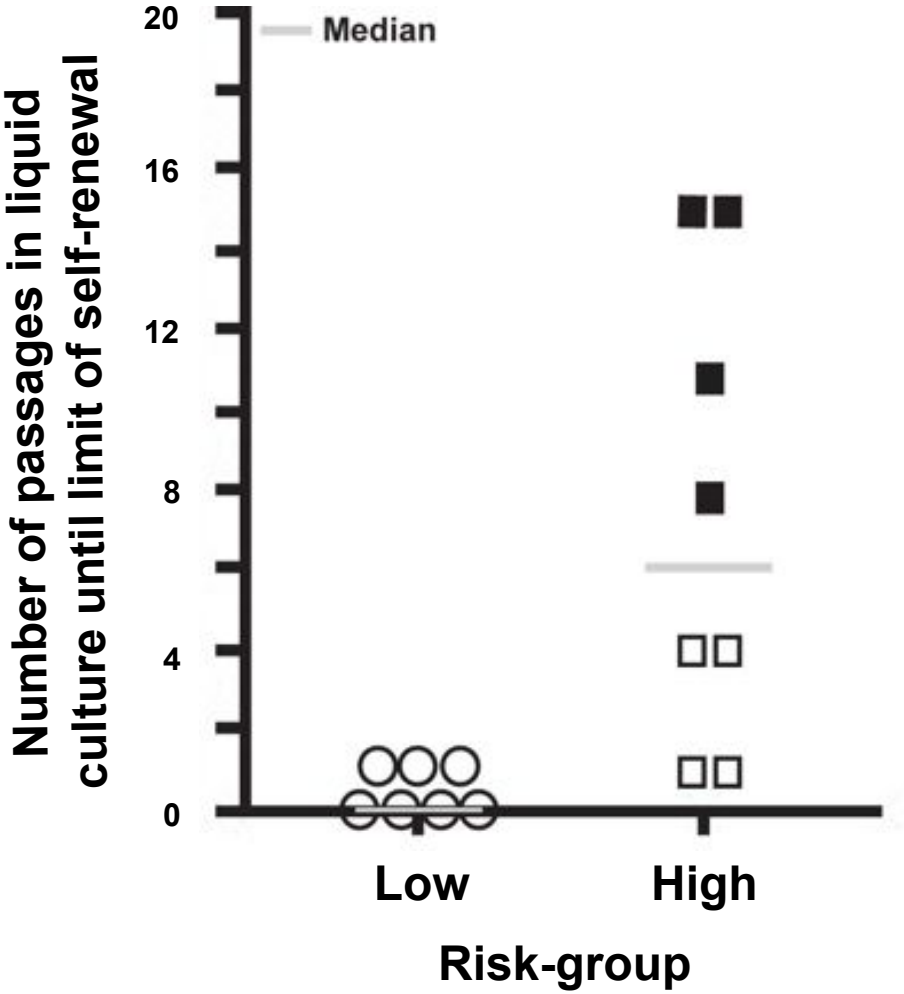
Stage 4 NB (high-risk) bone marrow



Nestin (neural crest stem cell marker)

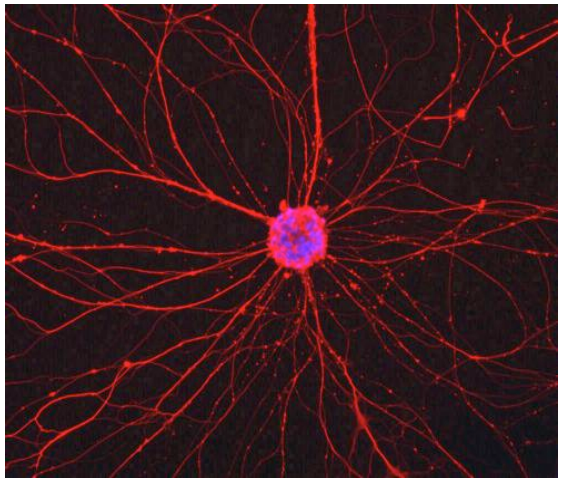


High-risk NB tumor spheres can be expanded and self renew

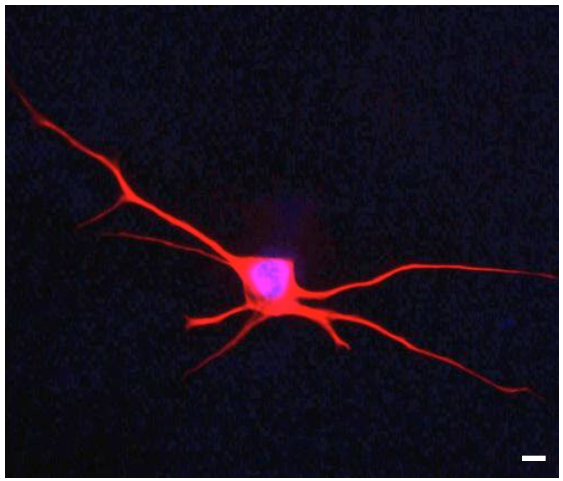


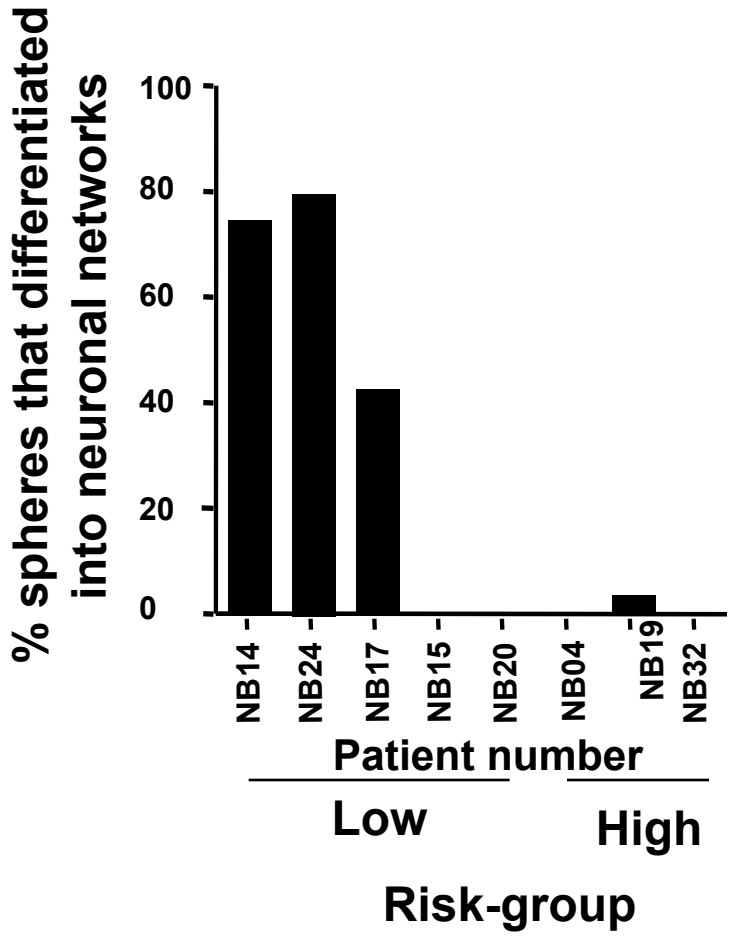
NB tumor spheres from low-grade NB differentiate into neurons

ßIII-tubulin (low-risk)



Nestin (high-risk)

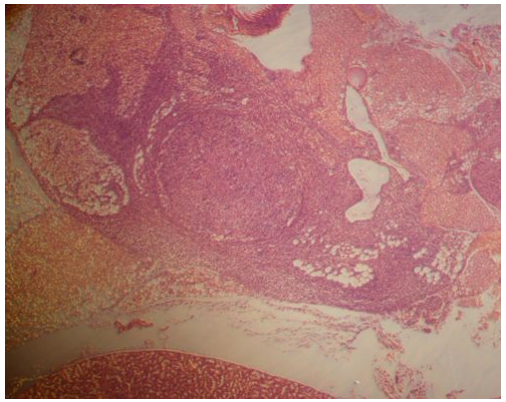




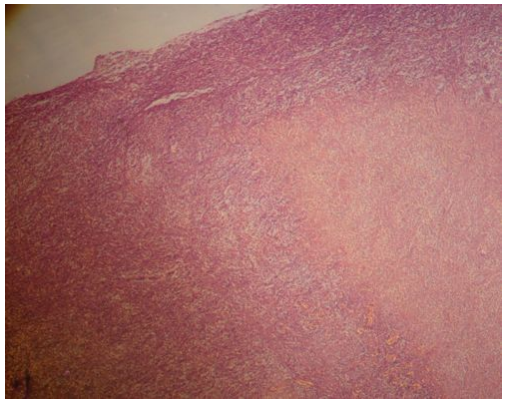
NB sphere-forming cells form neuroblastomas in mice that metastasize

NB TIC

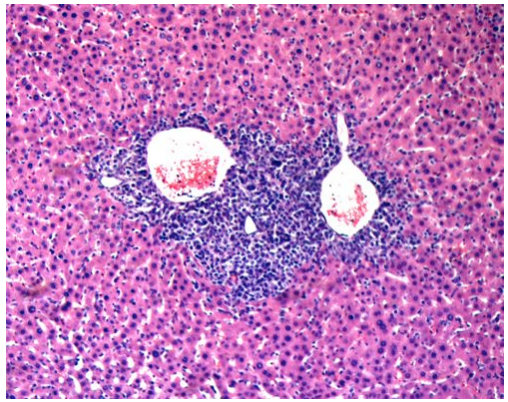
Micro-tumor



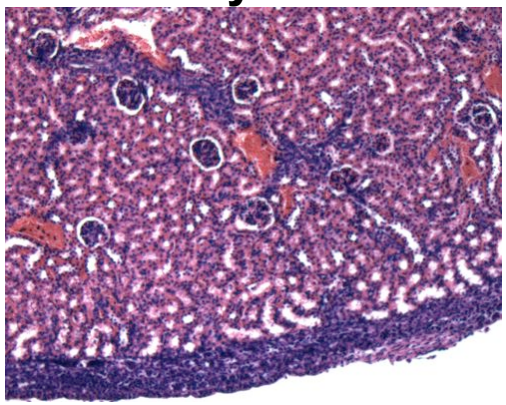
Tumor



Liver Metastasis



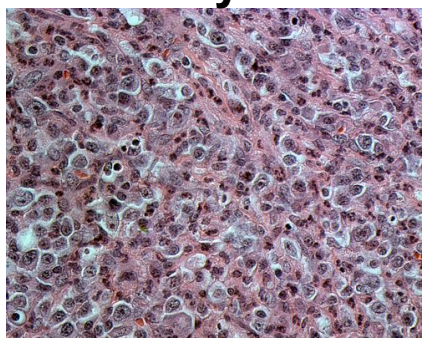
Kidney Invasion



Serial passage of tumors

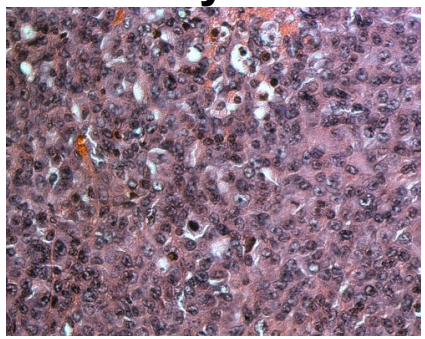
NB TIC

Primary Tumor

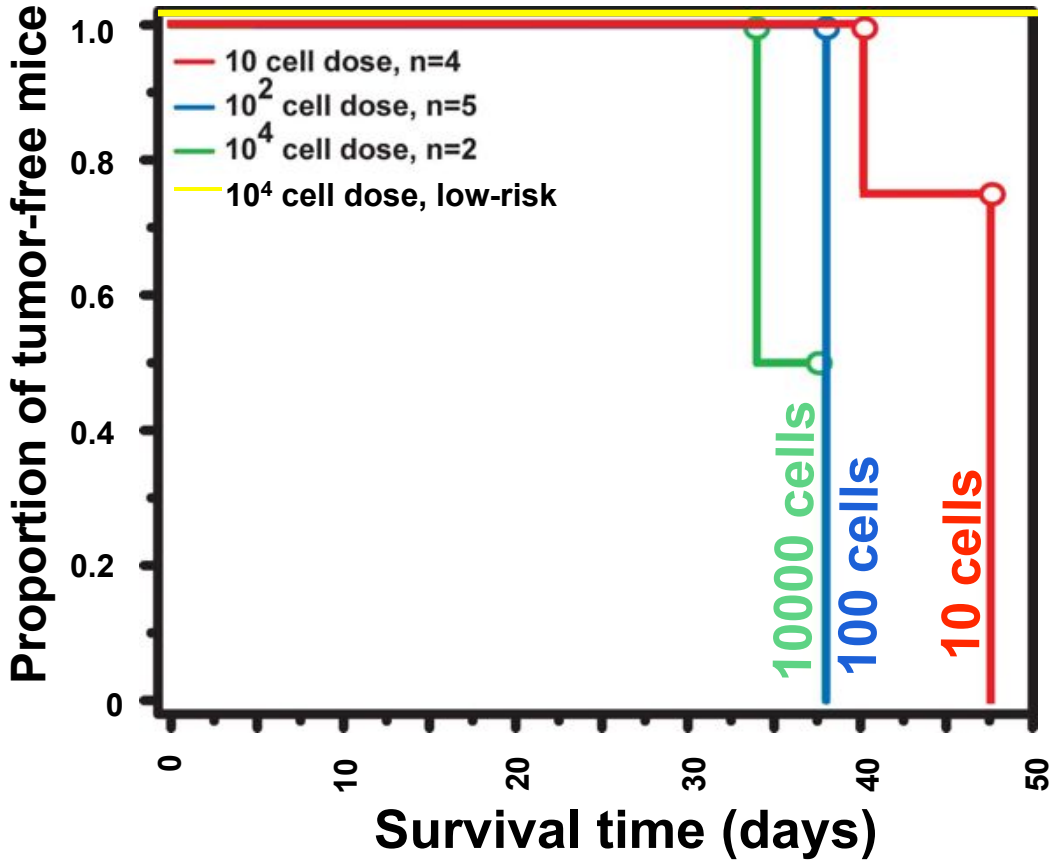


NB TIC

Tertiary Tumor

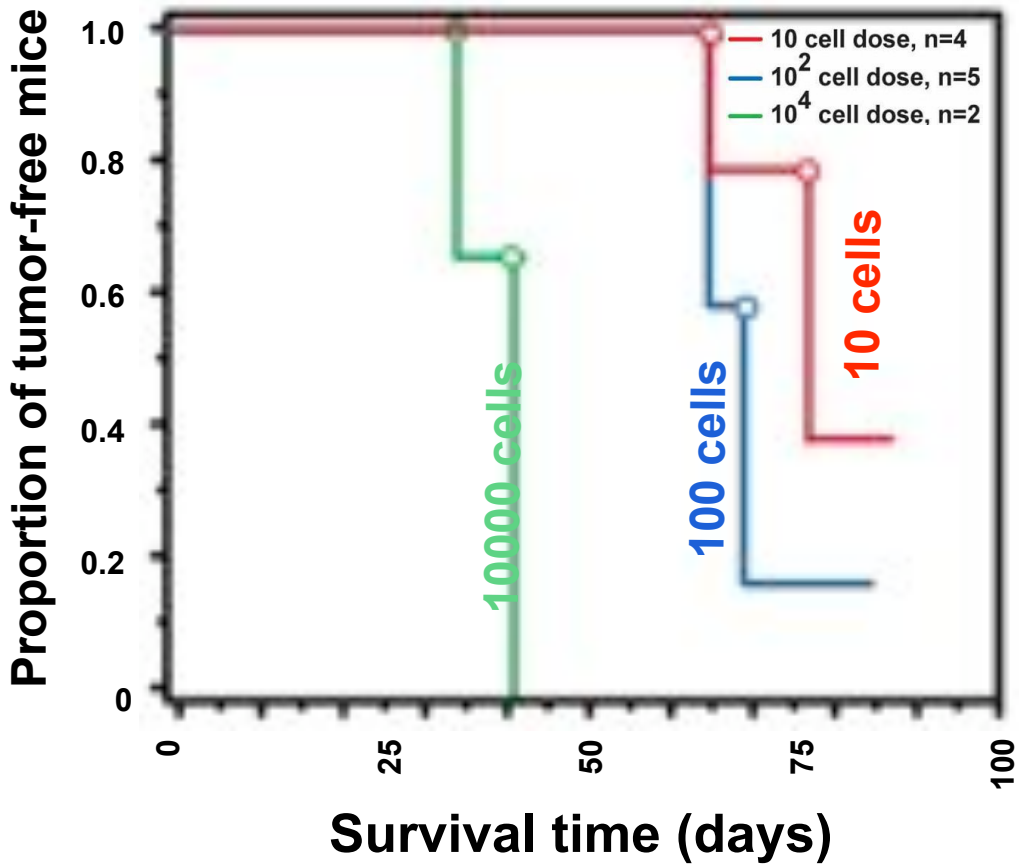


NB sphere-forming cells from high-risk patients form neuroblastomas in scid/beige mice at 10 cells



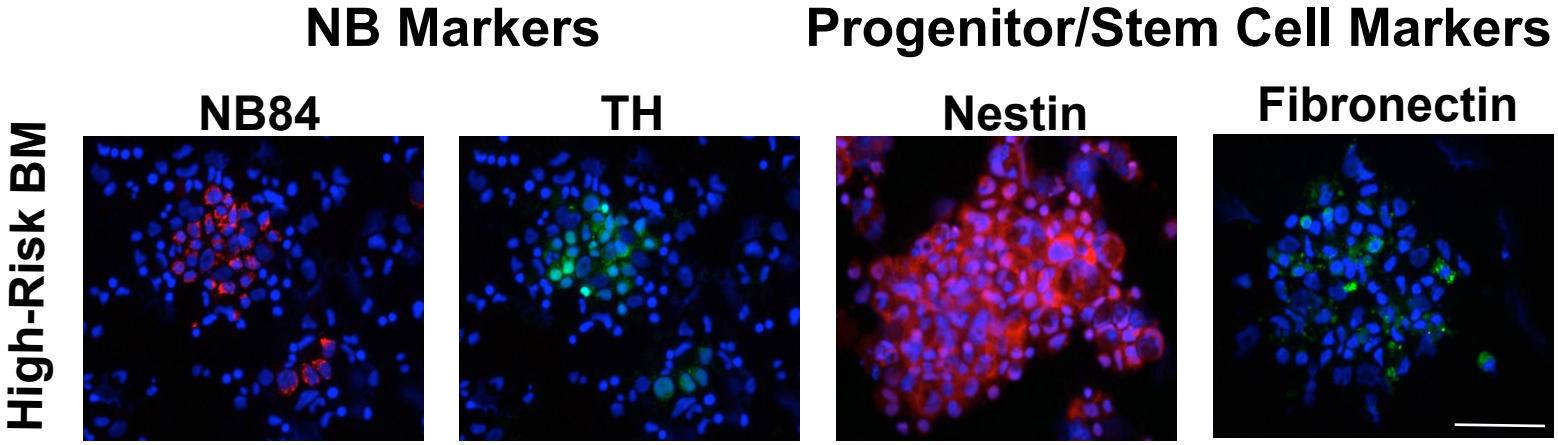
Note: primary tumor cells from patients will form tumors only at 1 x 10^6 cells

NB tumors form in mice from spheres isolated from the bone marrow of patients in remission



This patient died several months later from relapsed NB in the bone marrow

What are NB TICs?



NB TICs from bone marrow metastases express:

- Vimentin, nestin, fibronectin (neural progenitor markers)
- CD45, CD34 (hematopoietic markers)
- CD44, CD24 (mesenchymal markers)
- p75NTR, TrkB, NB84 (Schwann cells, neurons, neuroblastoma cells)

We propose: TICs are transformed early neural crest precursors that metastasize to the bone marrow, and acquire some characteristics of hematopoietic cells

Is there a neuroblastoma stem cell?

Is the reason why neuroblastoma often reoccurs due to a rare TIC that is resistant to chemotherapy?

Are NB TICs cancer stem cells?

In support:

- **Express neural stem/progenitor markers**
- **Self-renew multiple times in culture**
- **Differentiate into the cell types comprising NB**
- **TIC tumors can be serially passaged in mice**
- **Have highly enriched tumor-initiating capacity**

Against:

- **No hierarchy has been identified (every cell may be tumorigenic)**
- **No prospective marker identified**

Are NB TICs cancer stem cells?

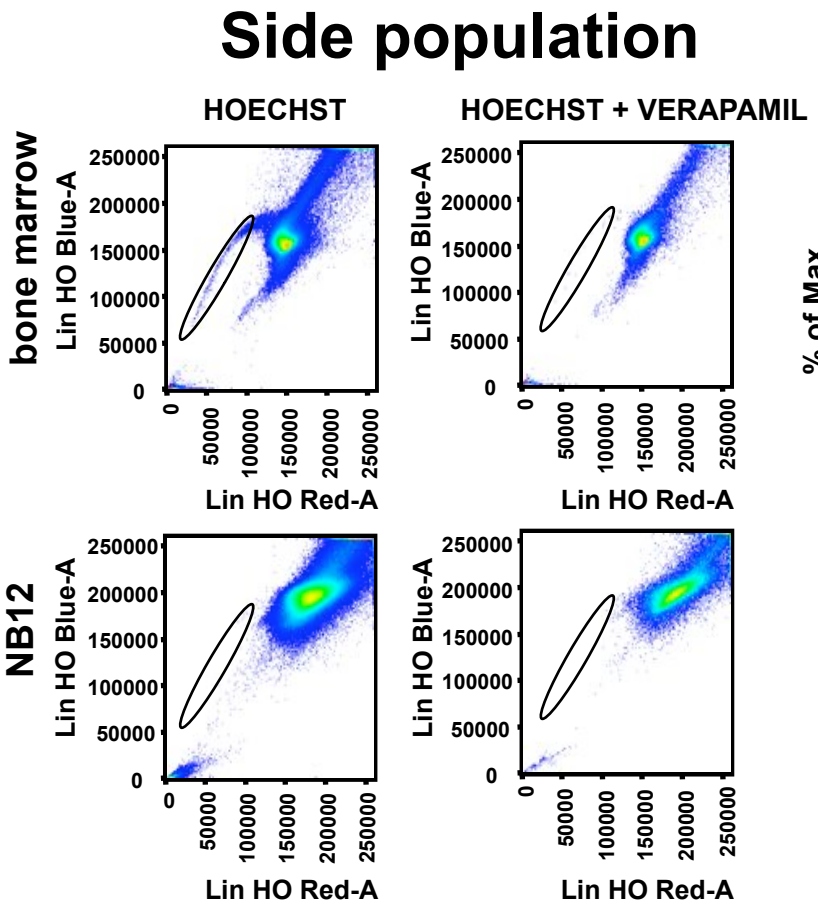
- Express neural stem/progenitor markers
- Self-renew multiple times in culture and as tumors
- Differentiate into the cell types comprising NB
- Have highly enriched tumor-initiating capacity

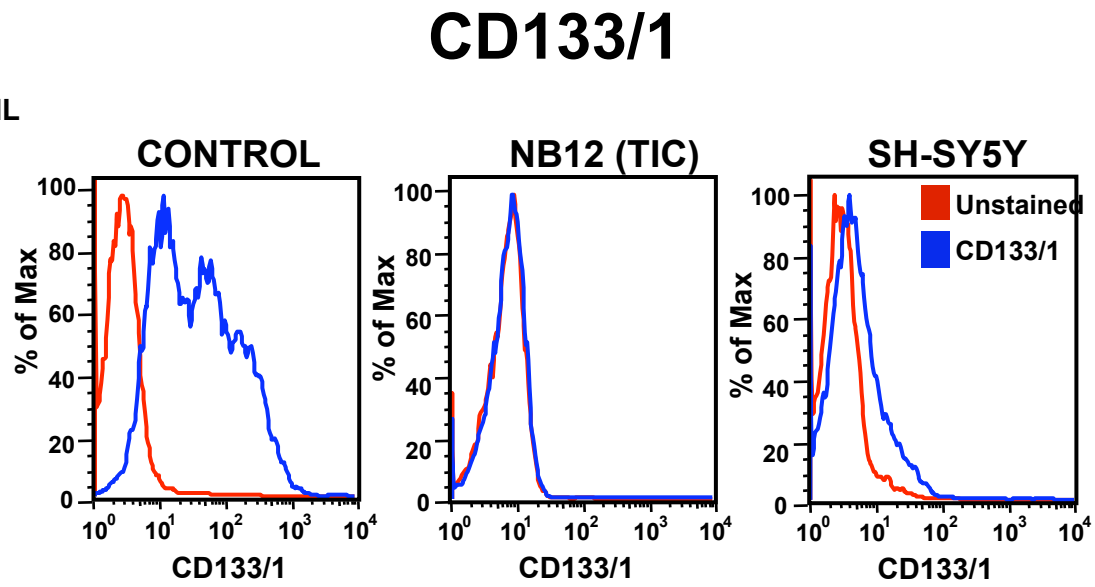
We use these cells to:

- identify NB oncogenes/tumor suppressors
- identify efficacious drugs (there are none)

...by comparing NB TICs to normal pediatric neural crest-like stem cells

NB TICs do not express CD133/1 or have a side population of cells

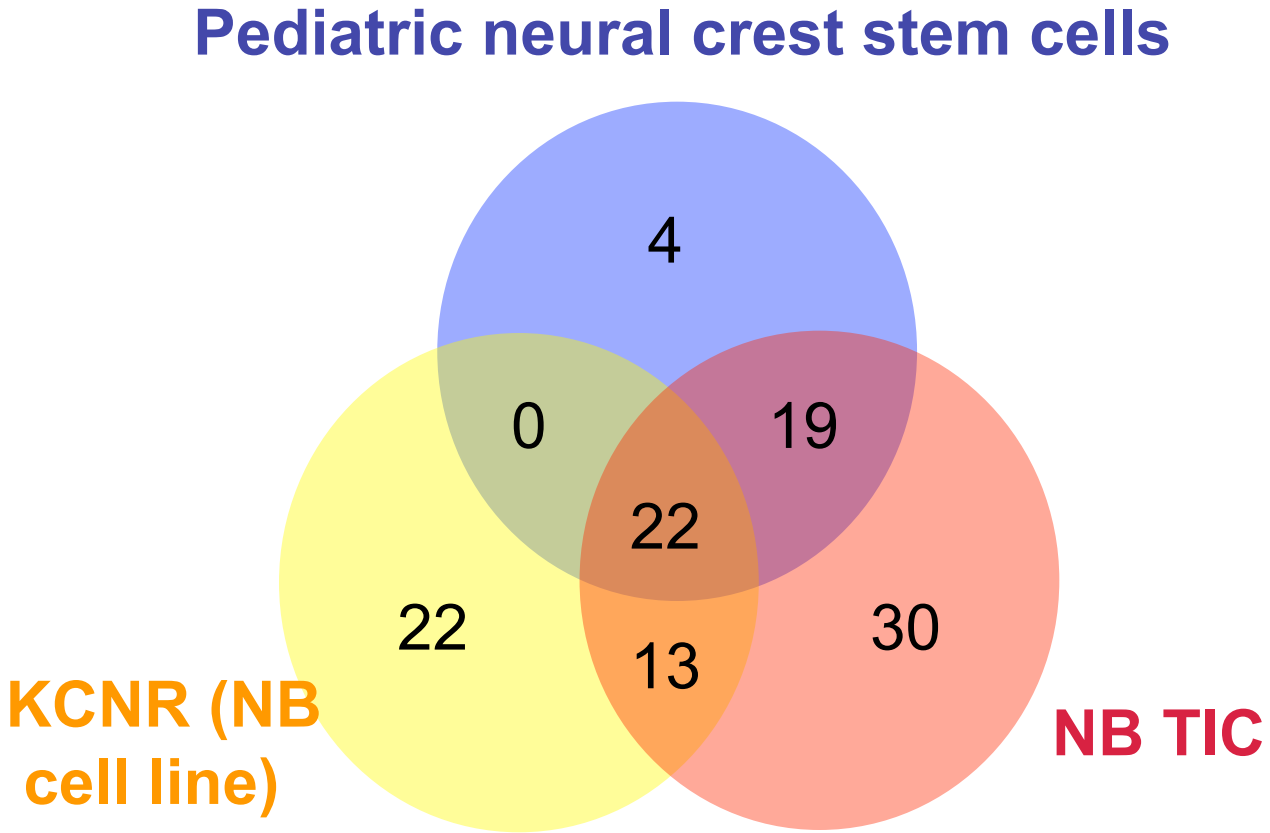




We predict 2 populations
in BM of relapsed patients
1. SP, CD133^{low}, GD2
2. non-SP, NB84, very tumorigenic

Loen Hansford

Primary NB TICs and a cell line respond very differently to cytotoxic drugs



Kristen Smith

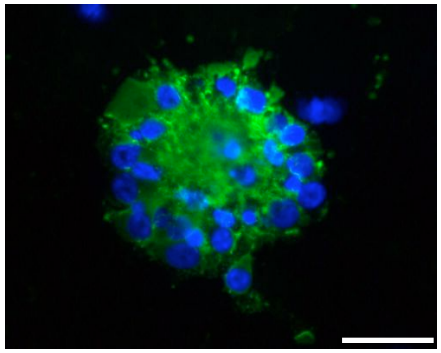
Does it matter if there is a neuroblastoma stem cell?

No, as long as we have a cell that resembles NB genetically and phenotypically, and that has a very high tumor-initiating capacity

drug discovery

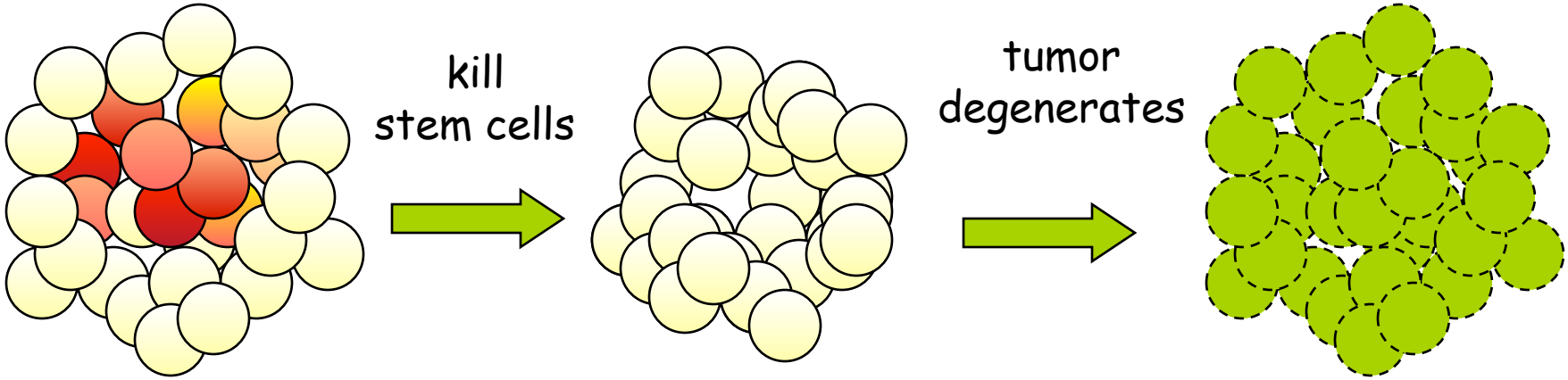
Is there an advantage to using primary TICs?

- Low passage as spheres in stem cell media to avoid cytogenetic changes and maintain TIC phenotype
- Cells readily expandable from same patient's tumor and following remission and relapse
- Can be obtained from favorable prognosis patients
- May more closely resemble disease state



Questions for NB TICs and stem cells

- Are they prognostic?
- Can we identify a prospective marker or novel oncogenes?
- Can we prevent NB TICs from metastasizing or homing to their niche
- Why do quiescent NB TICs in remission patients re-enter the cell cycle?
- Can we target NB TICs with drugs?



The Hospital for Sick Children,

Toronto

THE JAMES FUND
For Neuroblastoma Research at Sick Kids

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Loen Hansford -TIC

Kristen Smith - drug discovery

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Libo Zhang, Herman Yeger - NB mouse models

Sylvain Baruchel, oncologist, NB mouse models, new agents clinical trials

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