Profiling the Epigenetic Landscape of Breast Cancer Stem Cells

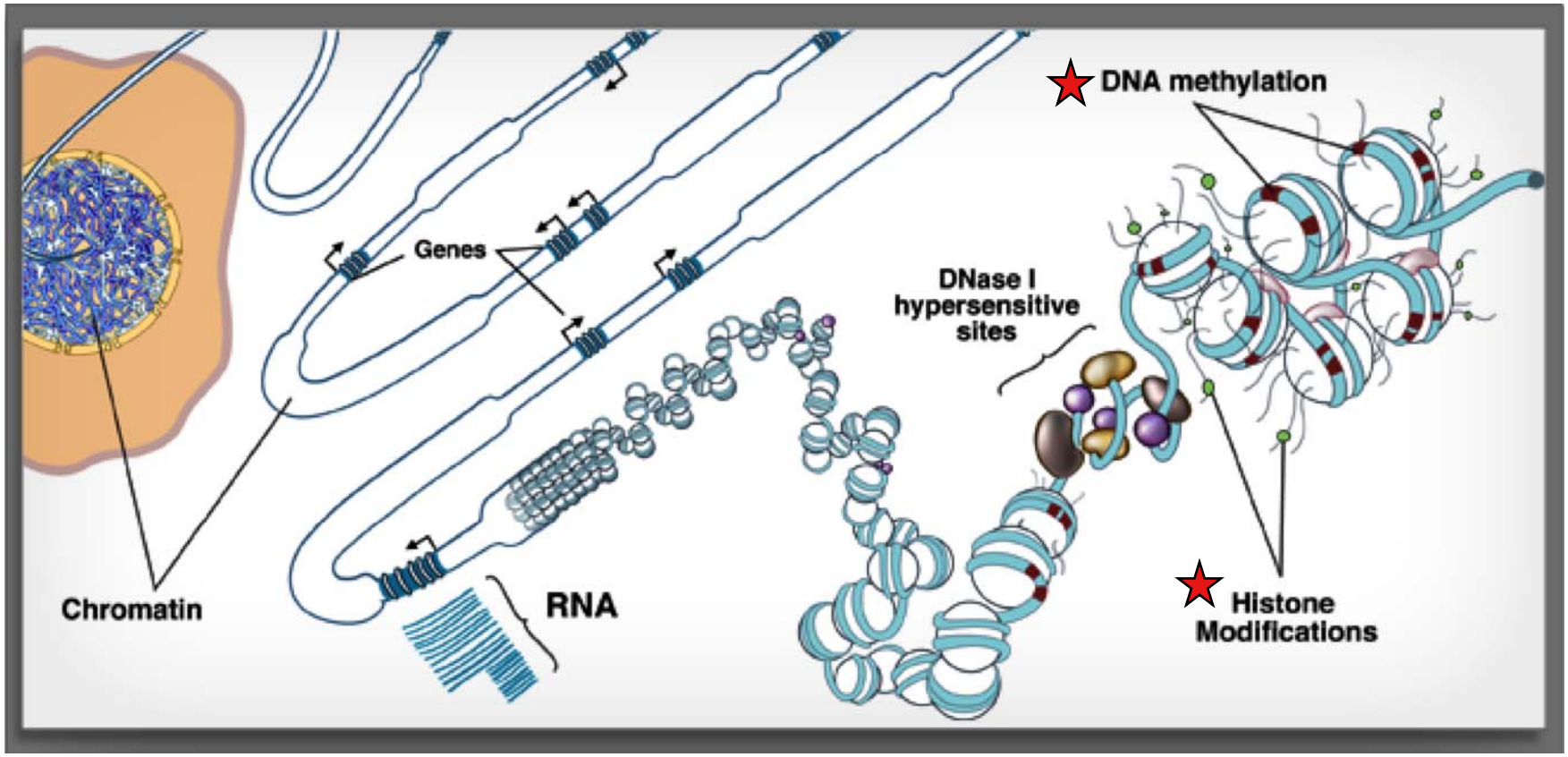
2010 GSC Retreat

Team - Epitome of Epiphany

29 November 2010

Background – Epigenetics

Epigenetics - the study of changes in the regulation of gene activity that are not dependent on DNA sequence



Background – Stem Cells

- Definition of a stem cell
  1. Self-renewal - the ability to go through numerous cycles of cell division while maintaining an undifferentiated state.
  2. High potency - the capacity to differentiate into specialized cell types.
- Scale of potency

Totipotent – produce all cells in an organism

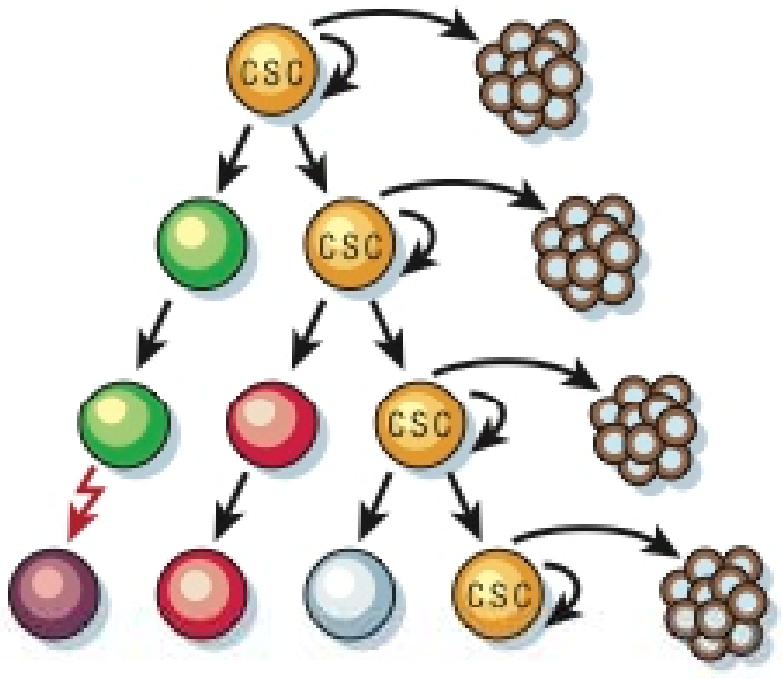
Pluripotent – produce almost all cells

Multipotent – produce one family of cells

Unipotent– only produce their own cell type

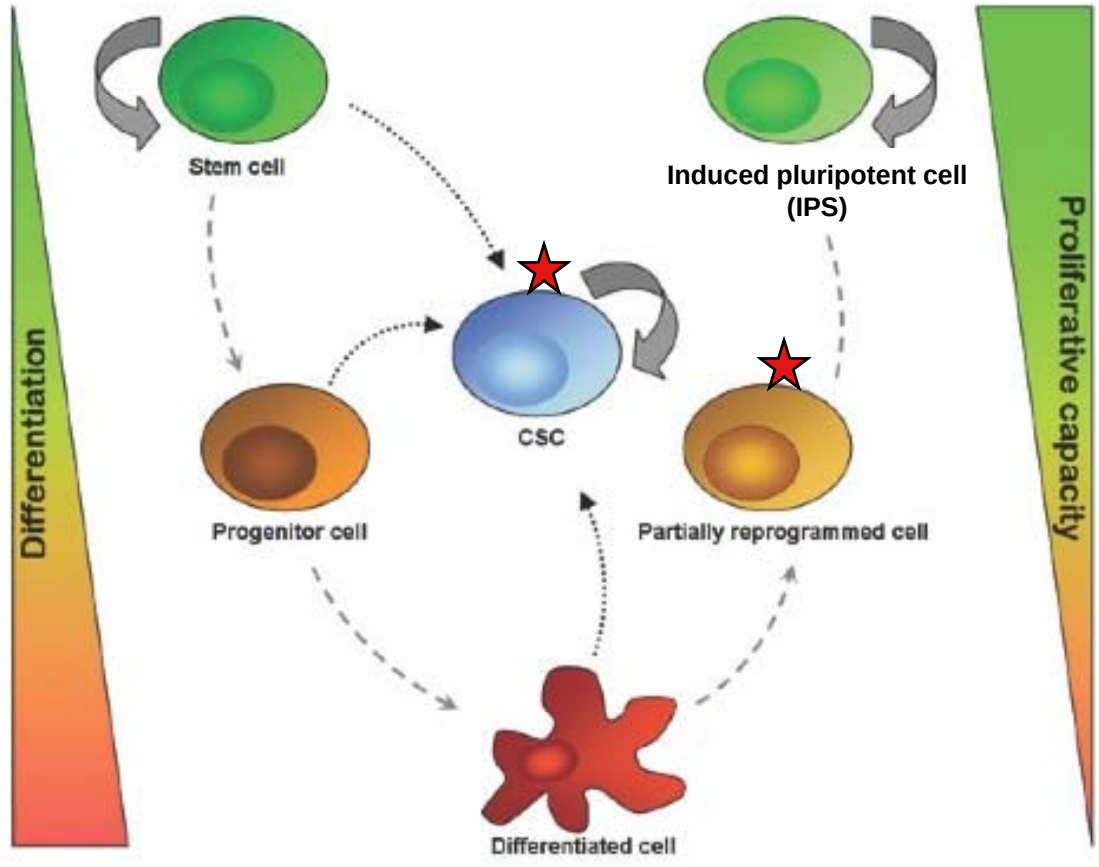
Background – Cancer Stem Cells (CSCs)

- Self renewal
- CSCs give rise to all cell types found in a particular cancer sample (i.e. heterogeneity)
- Only CSCs form new tumours



Reya et al. 2001 *Nature* 414:105-111.

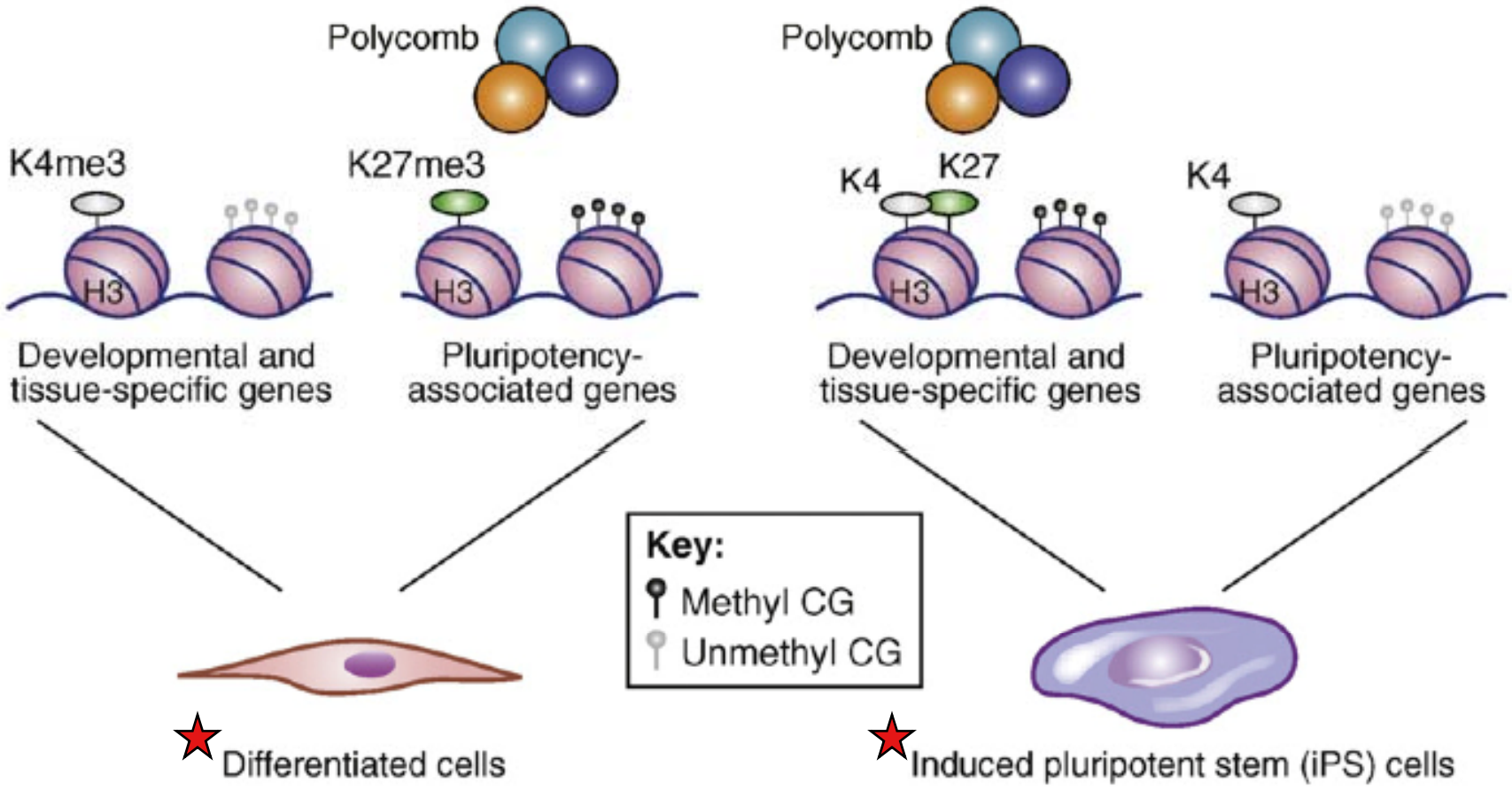
Background – Breakthroughs in stem cell research provide insight into cancer stem cells



- CSCs are similar to partially reprogrammed somatic cells

Welte et al. 2010 *Cell Communication and Signaling* 8:6

Background – Stem cells and differentiated cells show distinct epigenetic signatures



Amabile and Meissner 2009 *Trends in Molecular Medicine* 15:59-68

Hypothesis

- *Differences in the epigenetic landscape between breast CSCs and non-CSCs can be distinguished.*
  - *CSCs (CD44+/CD24-) vs. non-CSCs (remainder)*

- *The epigenetic programming acquired by CSCs can be modified toward differentiation, which could be exploited as a cancer-treatment strategy*.

Aims

1. Profile the differences in the epigenetic landscape between breast CSCs and non-CSCs derived from breast cancer cell lines

2. Profile the differences in the epigenetic landscape between breast CSCs, non-CSCs, and matched normal tissue isolated from patient primary tumours

3. Show that manipulation of the epigenetic landscape can change the cancer stem cell to a less tumourigenic/differentiated epigenetic state

Cell Lines

Following cell lines were selected based on 3 criteria:

1. Population presenting the stem cell markers
2. Tumourigenicity
3. Current available genomic data.

Cell lines/Criteria	$CD44^{+}/CD24^{-/low}$	Tumourigenicity	Genomic data
MDA-MB-231	+++	++	√
HCC1937	++	++	√
Hs578T	++	+	√

(+) 15-20% (++) 30-70% (+++) >70%

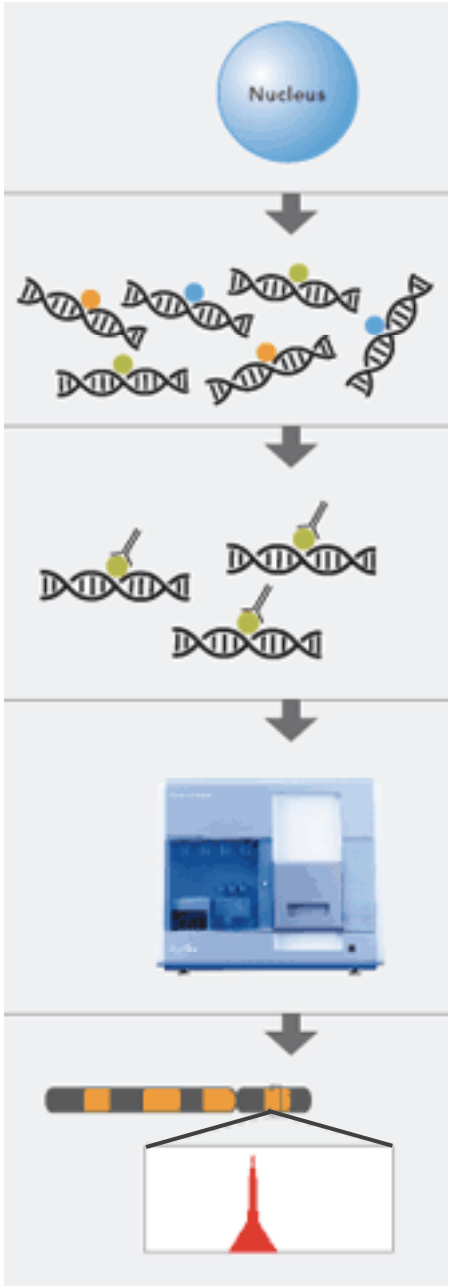
Hughes, et al. (2008). Clin Exp Metastasis 25, 549-557.
Hwang-Verslues, et al. (2009). PLoS One 4, e8377.
http://www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=sample&id=749714
http://www.sanger.ac.uk/genetics/CGP/Genotyping/nci60.shtml

Methods

ChIP-Seq
Chromatin
Immuno**p**recipitation and
Sequencing
for histone modifications

MeDIP-Seq
Methylated **D**NA
Immuno**p**recipitation and
Sequencing
for methylated DNA

MRE-Seq
Methylation-sensitive
Restriction **E**nzyme and
Sequencing
for unmethylated DNA



Cross-link and fractionate chromatin

ChIP - enrich for targeted binding sites

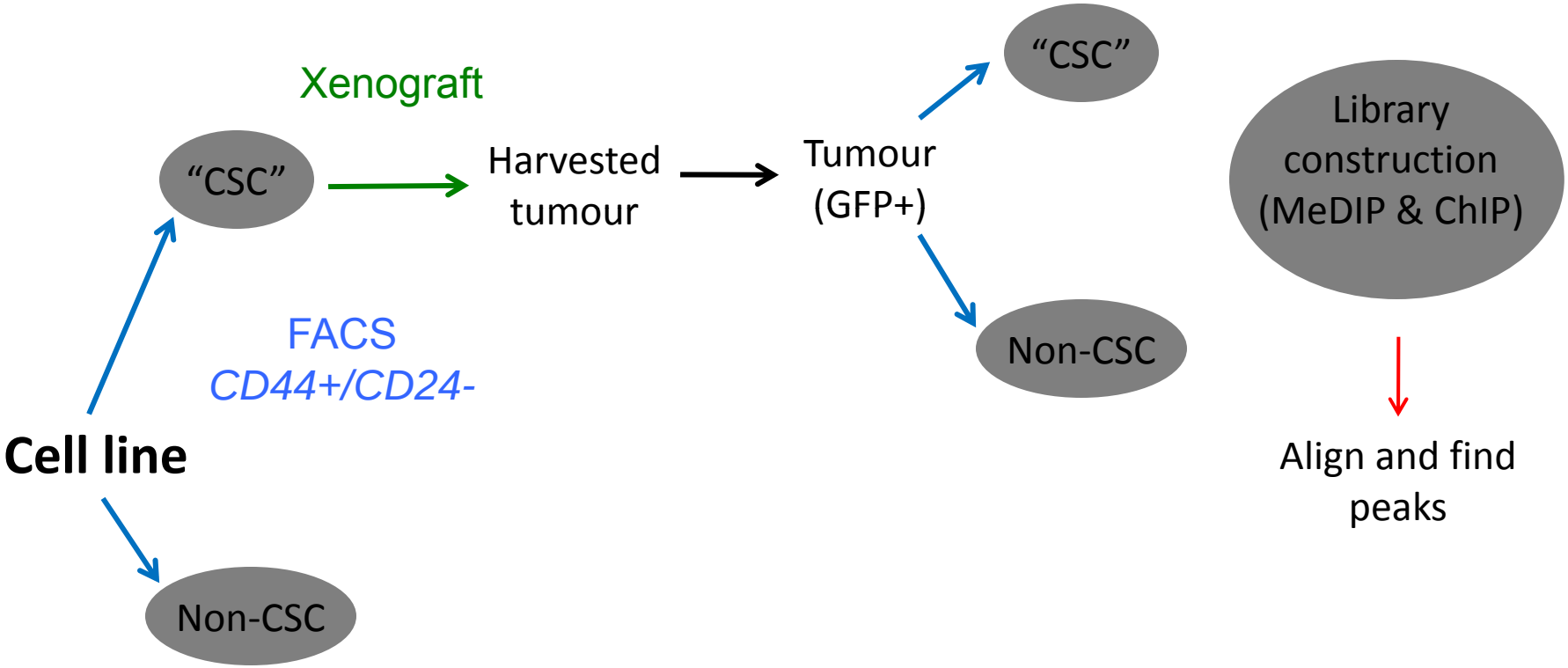
Sequence

Map sequence to reference genome

Peak = putative binding site

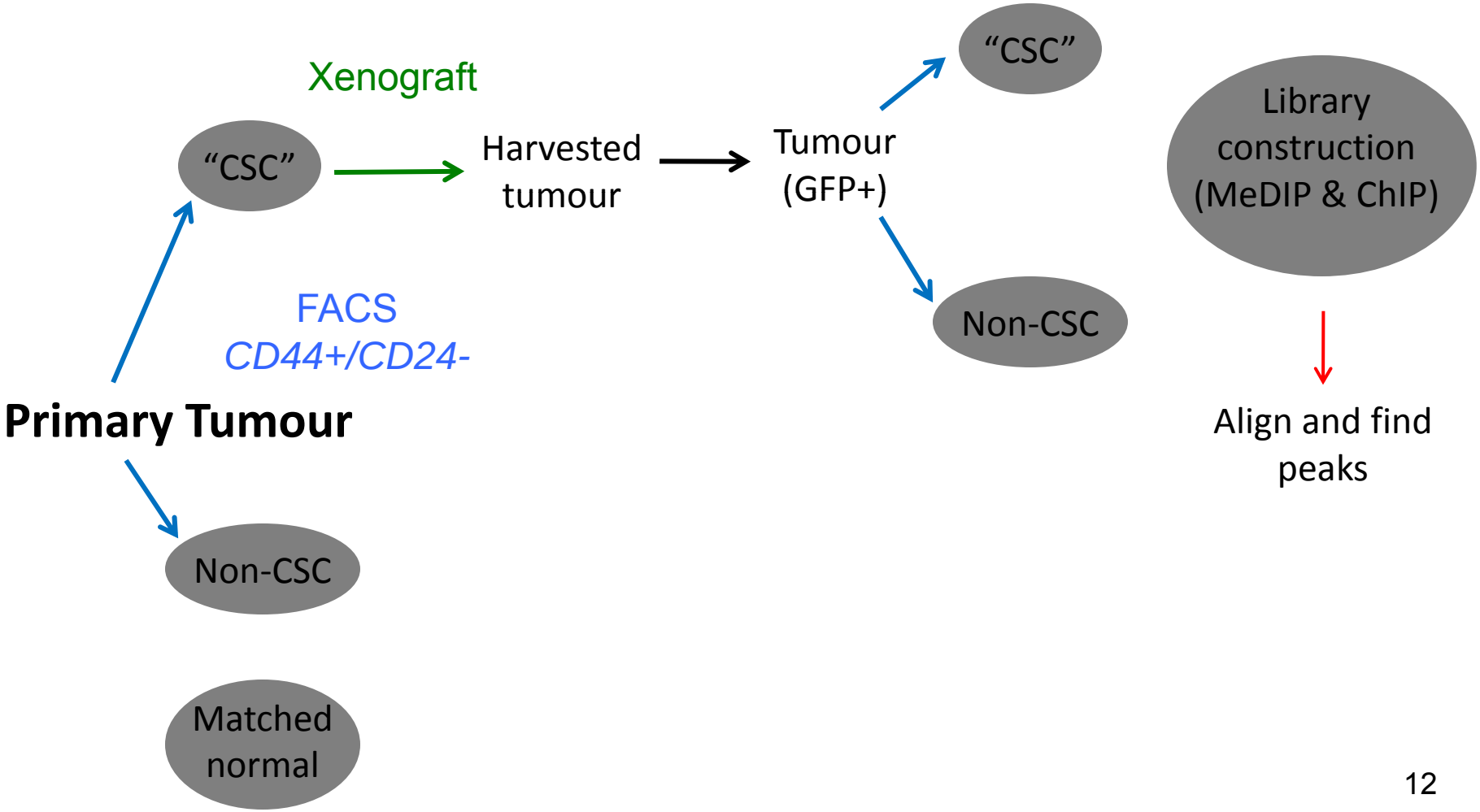
Methods

Aim 1. Profiling the epigenetic landscape of breast cell line derived CSCs and resulting bulk tumour.



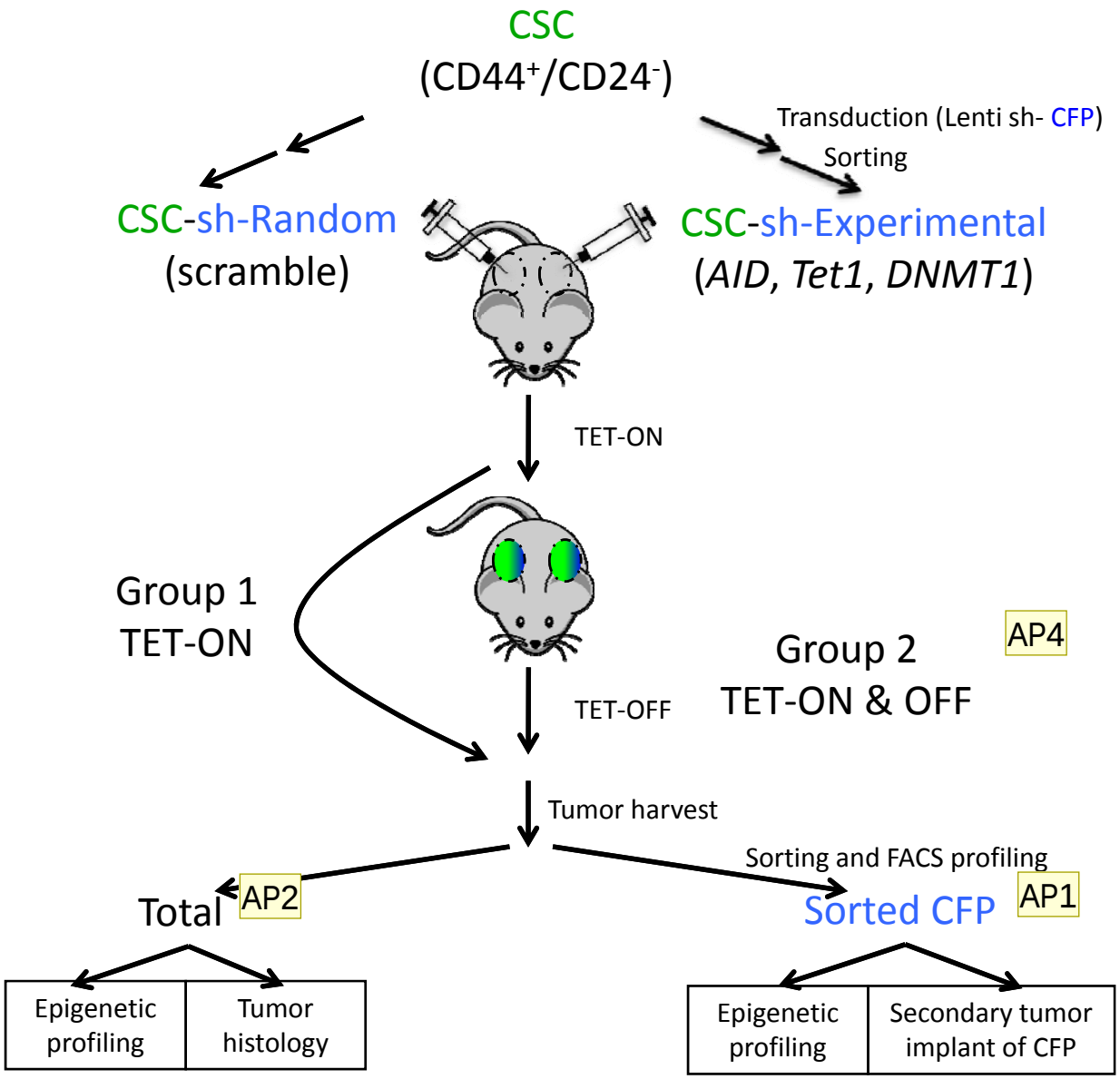
Aim 2. Profiling the epigenetic landscape of primary breast tumour CSCs and the resulting bulk tumour.

Methods



Aim 3. Manipulation of CSC epigenetic landscape by shRNA

Methods



Strategies and Rationales
What epigenetic reprogramming signals can we use?

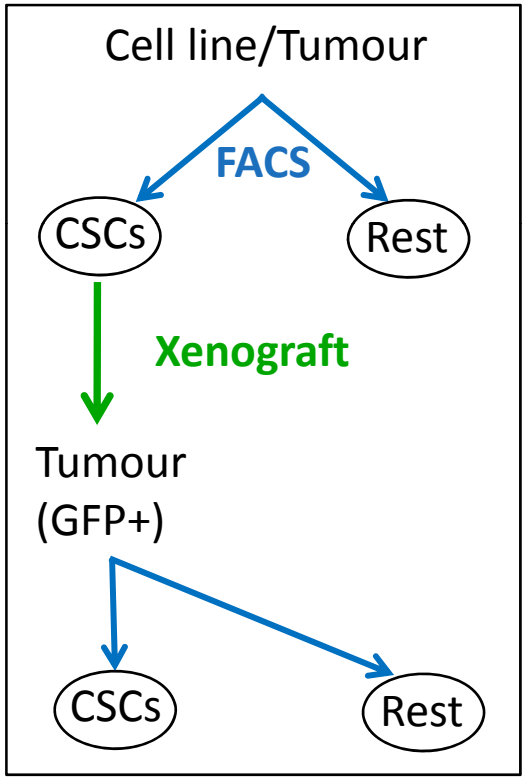
Downregulation of demethylase by shRNA

- *Blockade of AID (activation-induced cytidine deaminase) by shRNA* was shown to inhibit reprogramming of induce pluripotent cells (IPSC)
- *Blockade of Tet1 by shRNA* was shown to inhibit embryonic stem cell programming maintenance.

Downregulation of methylase by shRNA

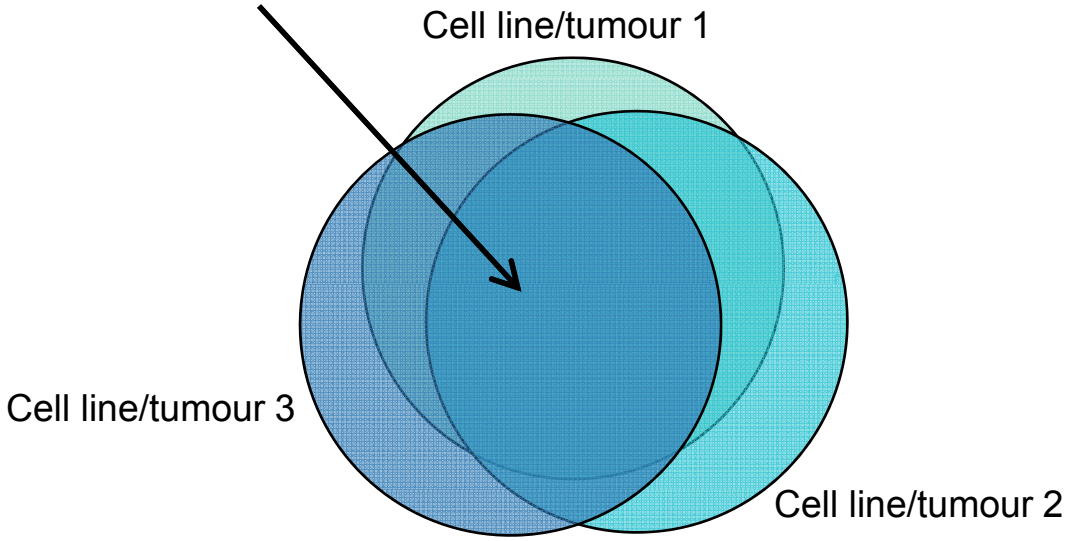
- *Blockade of DNMT1 by shRNA* has been shown to decrease breast cancer tumorigenicity.

Analysis: What are the recurrent epigenetic changes in CSCs?

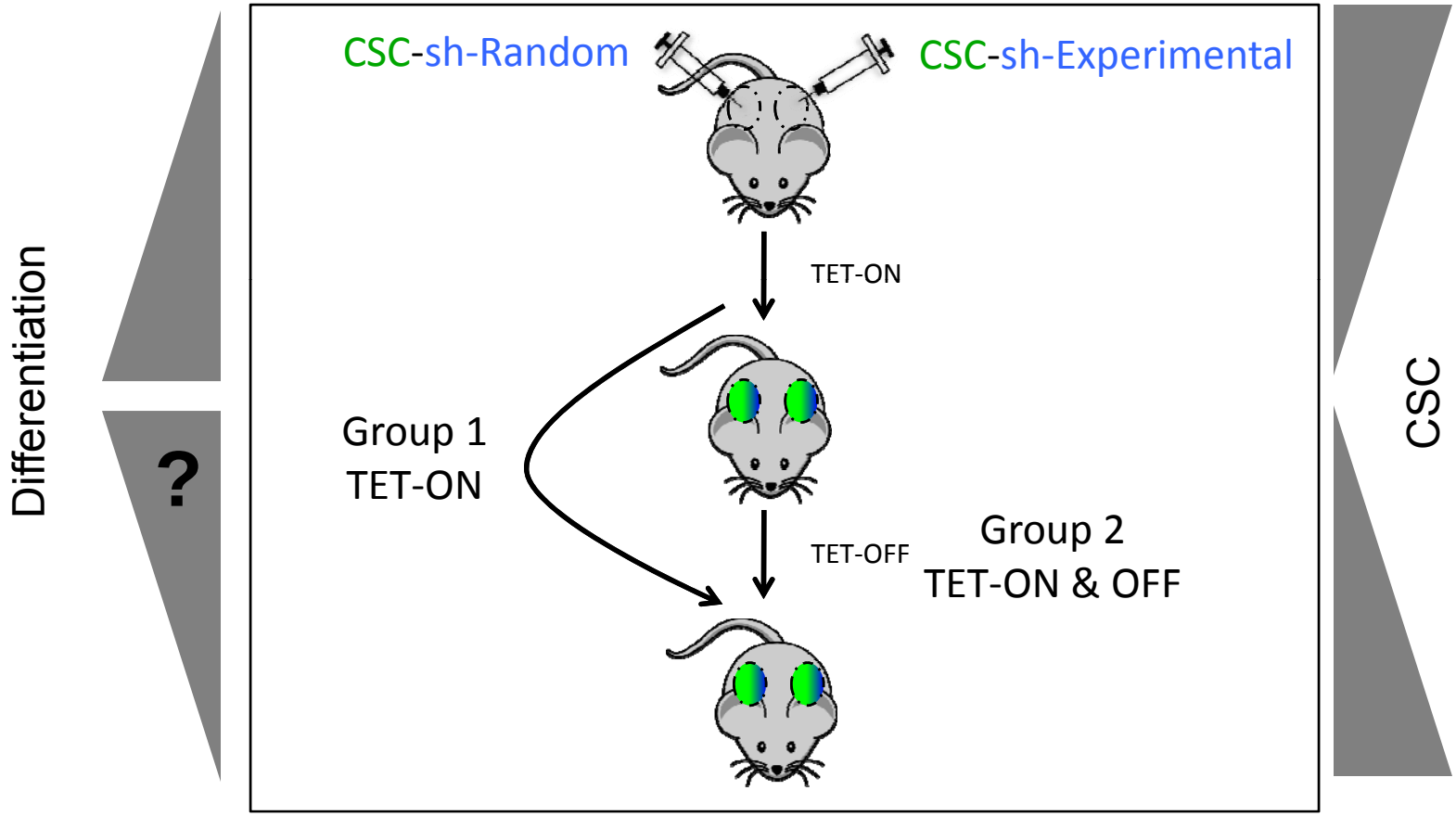


Epigenetic changes between CSCs vs. non-CSCs

Goal: Identify recurrent epigenetic changes between the three cell lines/primary tumours



Analysis: Can CSCs be modified toward differentiation in vivo?



Expect to find enrichment for genes with CSC characteristic signatures showing changes during the transition of cells to a more differentiated state

AP6

Potential Problems

- Poor engraftment of the CSCs isolated from primary tumours.
- Tumour heterogeneity
- Genetic mutation

Significance

- CSCs are putatively the source of cancer recurrence and treatment resistance
- Provide a reference CSC epigenetic profile
- Understanding the modifications that contribute to CSC pluripotency may lead to the development of new therapies

Timeline (5 years)

Aim	Task	Year 1				Year 2				Year 3				Year 4				Year 5			
		Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4	Q 1	Q 2	Q 3	Q 4
Aim 1	Profile the differences in the epigenetic landscape between breast cancer stem cells derived from breast cancer cell lines and the resulting bulk tumor, using a mouse xenograft model																				
Aim 2	Profile the differences in the epigenetic landscape between breast cancer stem cells isolated from patient primary tumours, non-CSCs derived from the same tumor and normal tissue.																				
Aim 3	Show that manipulation of the epigenetic landscape by shRNA treatments of methylation regulating enzymes can change the cancer stem cell to a less tumourigenic/differentiated epigenetic state or vice versa.																				

Epitome of **Epi**phany Team Members

Mauro Castellarin

Grace Cheng

Malachi Griffith

Cydney Nielsen

Alex Patenaude

Johanna Schuetz

Iran Tavakoli