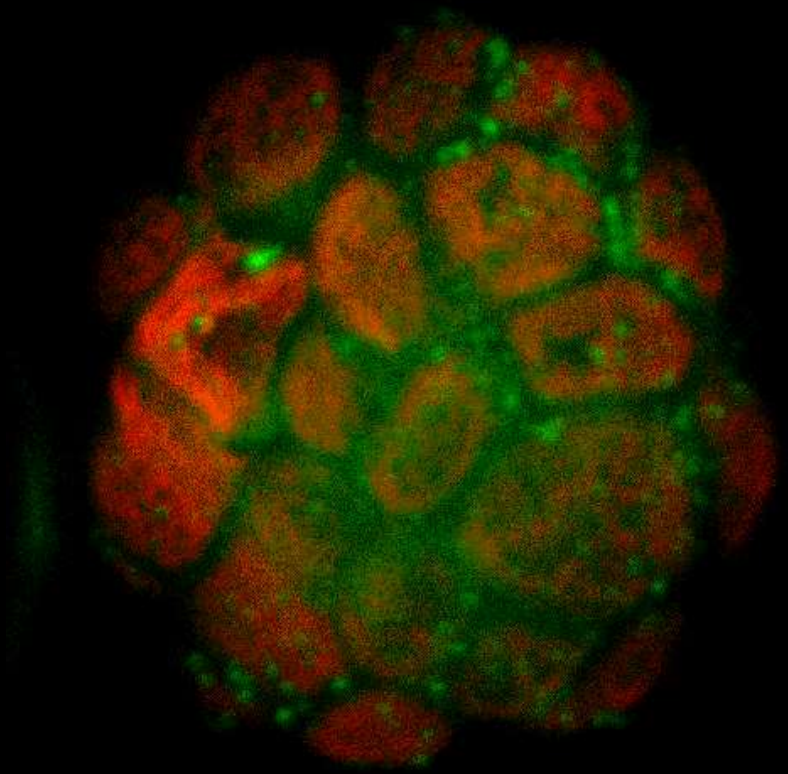
Cancer Stem Cells and stress induced evolution - understanding the drug recalcitrance phenomenon



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NCCS

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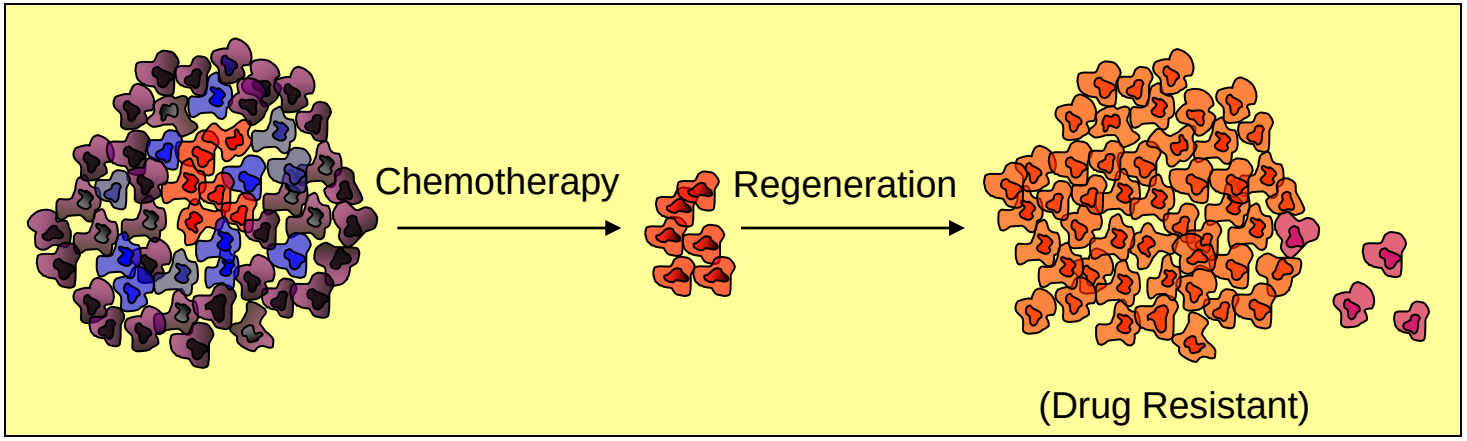
Post-therapy minimal residual disease – drug recalcitrance

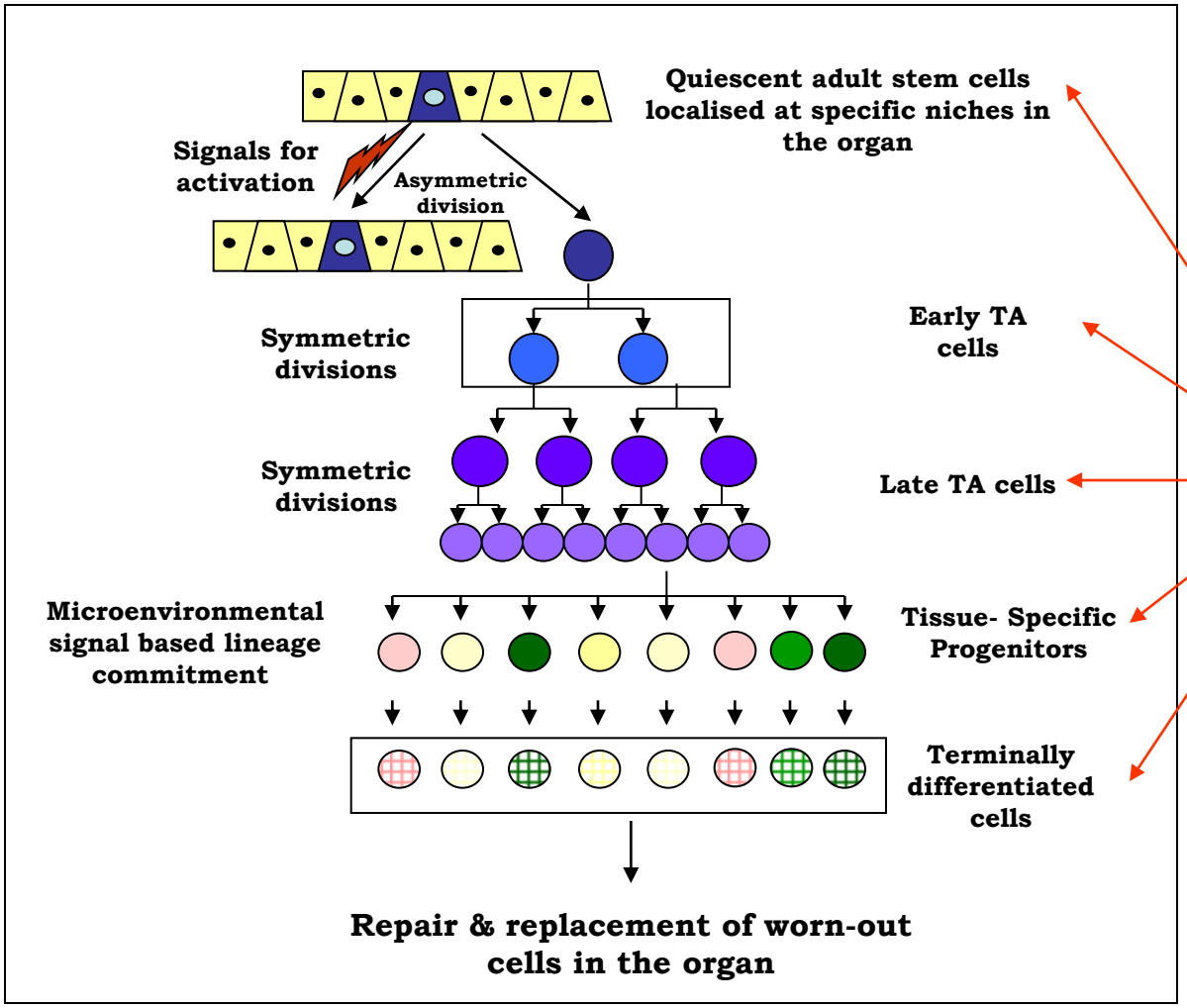


- Most therapies fail to consider differential drug sensitivities of various cells in a tumor (**Tumor Cell Heterogeneity**)

- Drug refractory behaviour of tumor cells may arise due to either –

  - *Intrinsic drug resistance mechanisms (**Molecular Heterogeneity**)*
  - *Cell dormancy / reversible quiescence (**Cancer stem cells - CSCs**)*

Stem cell hierarchies



Intra-tumor Heterogeneity

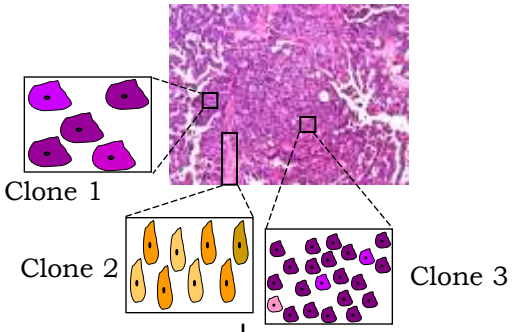
Metastases-driven

Dominance of different clones at different sites of metastases

Molecular & Functional

Differential gene and protein regulation in different cells

Cellular



Diverse subpopulations that often are considered to be homogeneous

(i) Regenerative Heterogeneity - Cancer Stem Cell hierarchy

(ii) Genetic Variation - Instability and Somatic Evolution

(iii) Transient heterogeneity during cell cycling

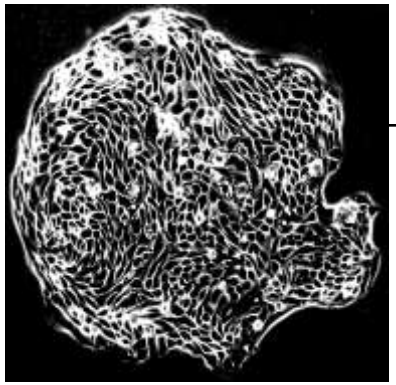
(iv) Functional heterogeneity

(v) Host cells

Effective therapy

Development of the ovarian cancer stem cell model

Patient Tumor Ascites



19 immortalized single cell clones

1 tumor initiating CSC clone

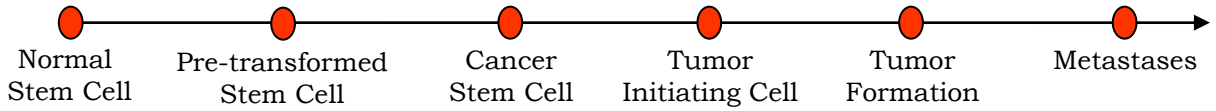
1 progression model – paired Pre- and post transformed clones

17 non-tumorigenic clones

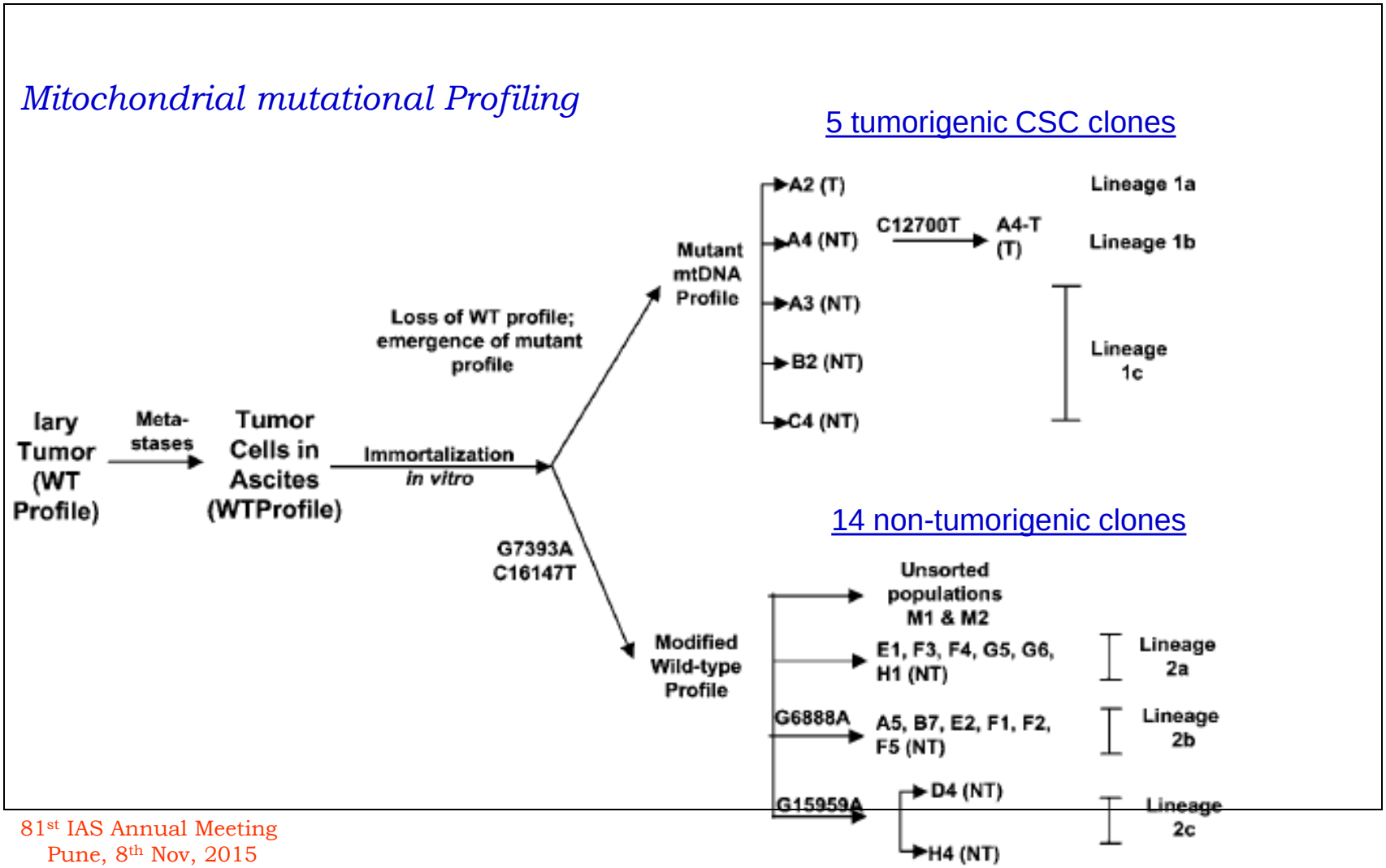
First report on isolation and identification of Ovarian cancer stem cells

Bapat et al. Cancer Res. 2005

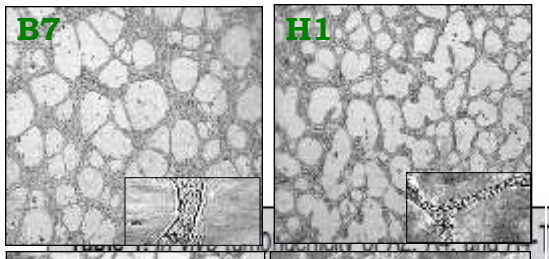
Genetic diversity in tumors



Mitochondrial mutational Profiling

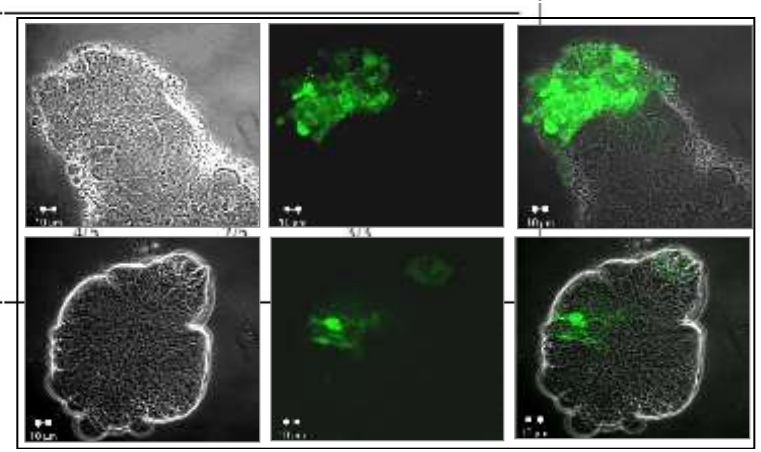


Functional diversity in tumors



Wild-type clones express CD133 – capable of endothelial differentiation

			cells		
			Ascites formation	Metastases	Mortality	Tumor formation on serial transplantation
	i.p.	4/4	—			2/4
A4 (P8)	s.c.	0/2	—			—
A4-T (P25-P28)	s.c.	6/6	—			—
	i.p.	5/5	3/5			

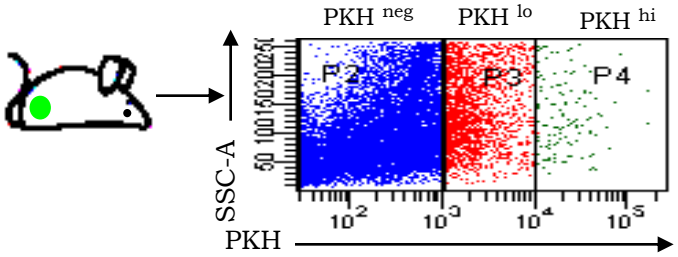


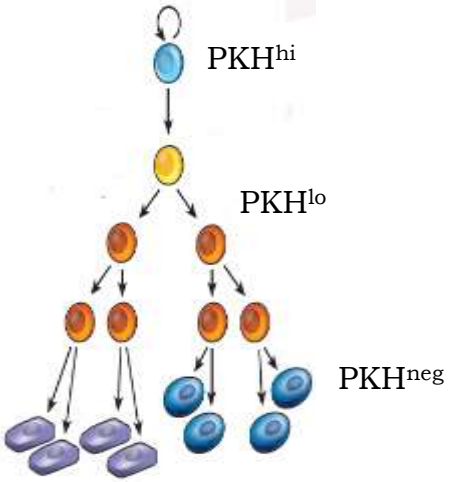
CD133 cells are recruited by CSCs to establish tumor vasculature through a definitive endothelial hierarchy

Kusumbe et al. Stem Cells. 2009

Resolution of tumor heterogeneity

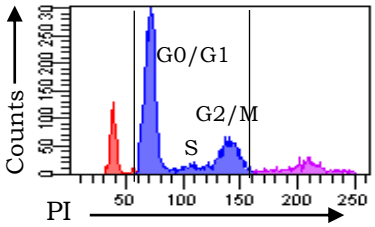
Cancer stem cell hierarchies : Label-chase



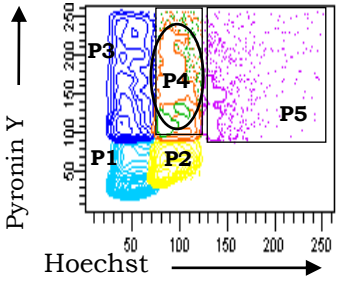


Host - Genetic instability: Aneuploidy (DNA Content)

Host	Eu	Aneu
12%	66%	22%



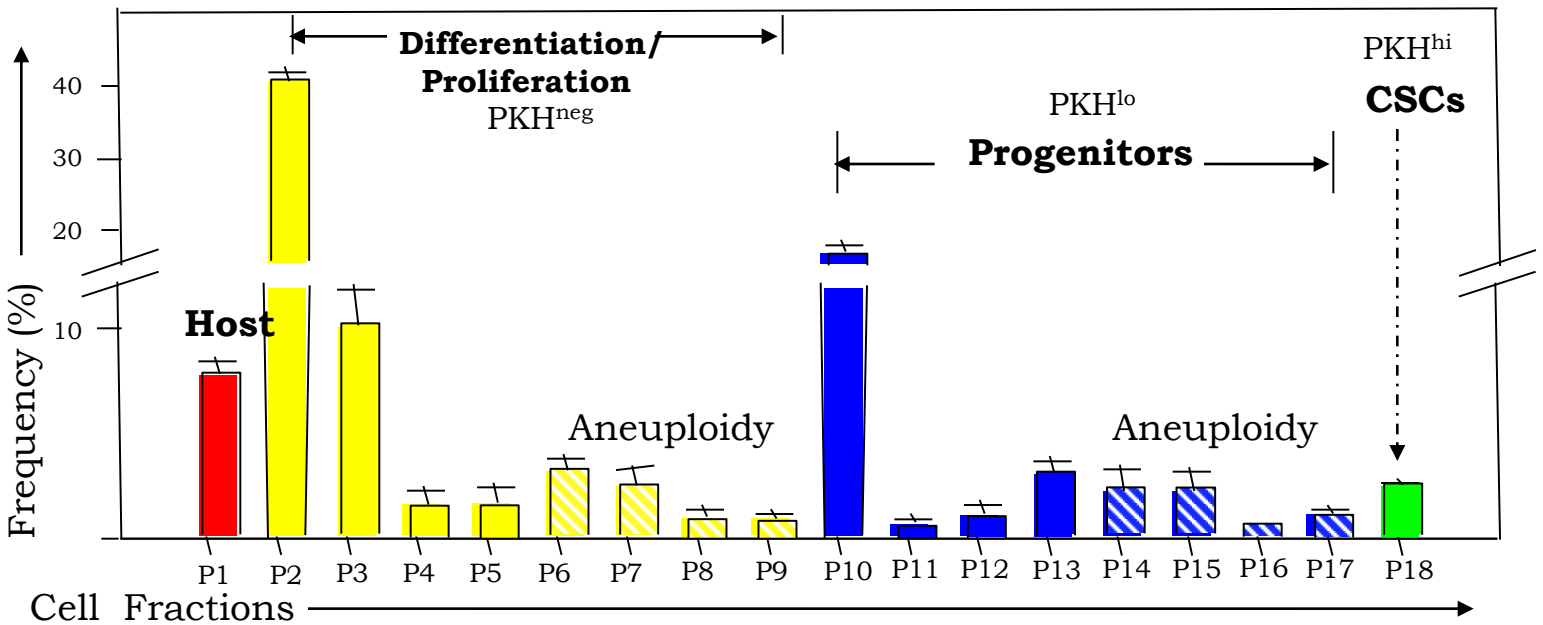
Cell Cycle phases : DNA – RNA content



Kusumbe & Bapat. Cancer Res. 2009

Deconstruction of a solid tumor – Defining its 'Cytotype'

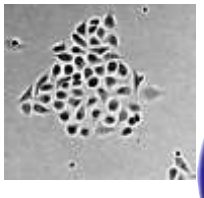
Regenerative hierarchy – Genetic instability - Cell Cycle - Functionality



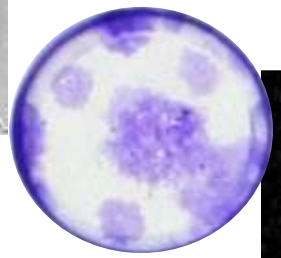
P1 - Host
P2 - PKHneg; Eu-S
P3 - PKHneg; Aneu-S
P4 - PKHlo; Eu-S
P5 - PKHlo; Aneu-S
P6 - PKHneg; Eu-G0
P7 - PKHneg; Eu-G1
P8 - PKHneg; Aneu-G0
P9 - PKHneg; Eu-G2M
P10 - PKHneg; Aneu-G1
P11 - PKHneg; Aneu-G2M
P12 - PKHlo; Eu-G0
P13 - PKHlo; Eu-G1
P14 - PKHlo; Aneu-G0
P15 - PKHlo; Eu-G2M
P16 - PKHlo; Aneu-G1
P17 - PKHlo; Aneu-G2M
P18 - PKHhi; Eu-G0

Naik et al. Oncogene 2015
Naik & Bapat - IPO Application 2014; PCT Application 2015

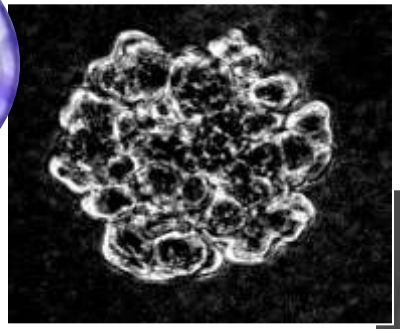
Functional Evaluation of Individual Fractions



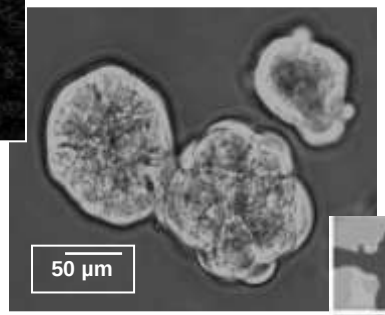
Adherent Colonies



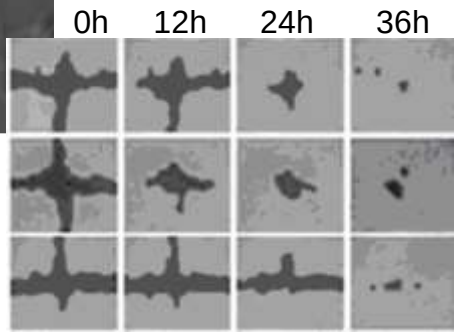
Colonies in soft agar



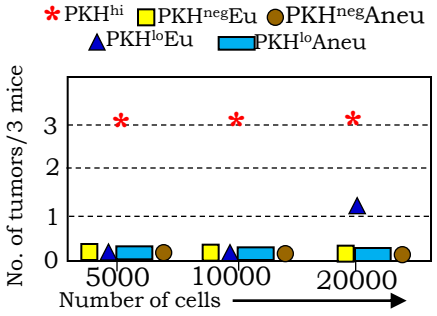
Spheroid Formation



Wound Healing



In vivo Tumorigenecity – TIC Potential

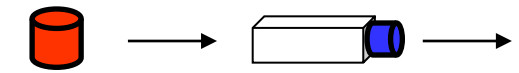


Tumor fractions	Tumor Initiating Frequency
$PKH^{neg}Eu$	-
$PKH^{neg}Aneu$	-
PKH^{lo} Eu	1/94648
$PKH^{lo}Aneu$	-
PKH^{hi} Eu	1

Using the tumor Cytotype to understand Drug Responses

Conventional Drug Evaluation

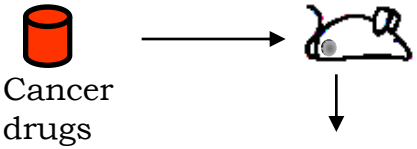
(i) In vitro



Cancer drugs

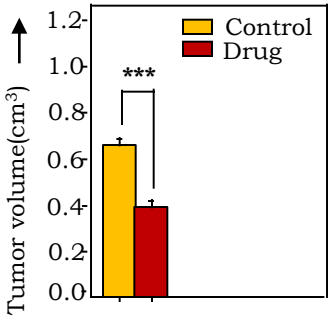
Cytotoxicity to relatively homogeneous cells

(ii) In vivo



Cancer drugs

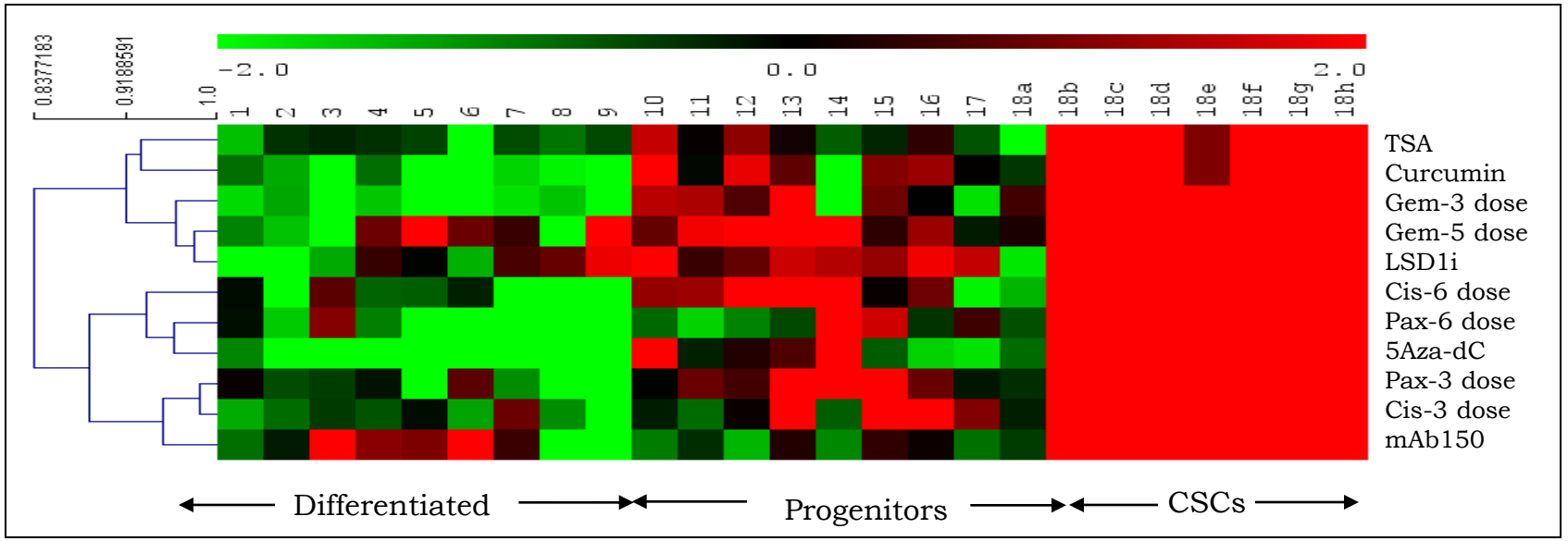
Tumor regression with limited analysis of residual tumor heterogeneity



Drug Resistant Cells - CSCs, mutations, aneuploidy

Tumor dormancy and reversible quiescence

Using the tumor Cytotype to understand Drug Responses



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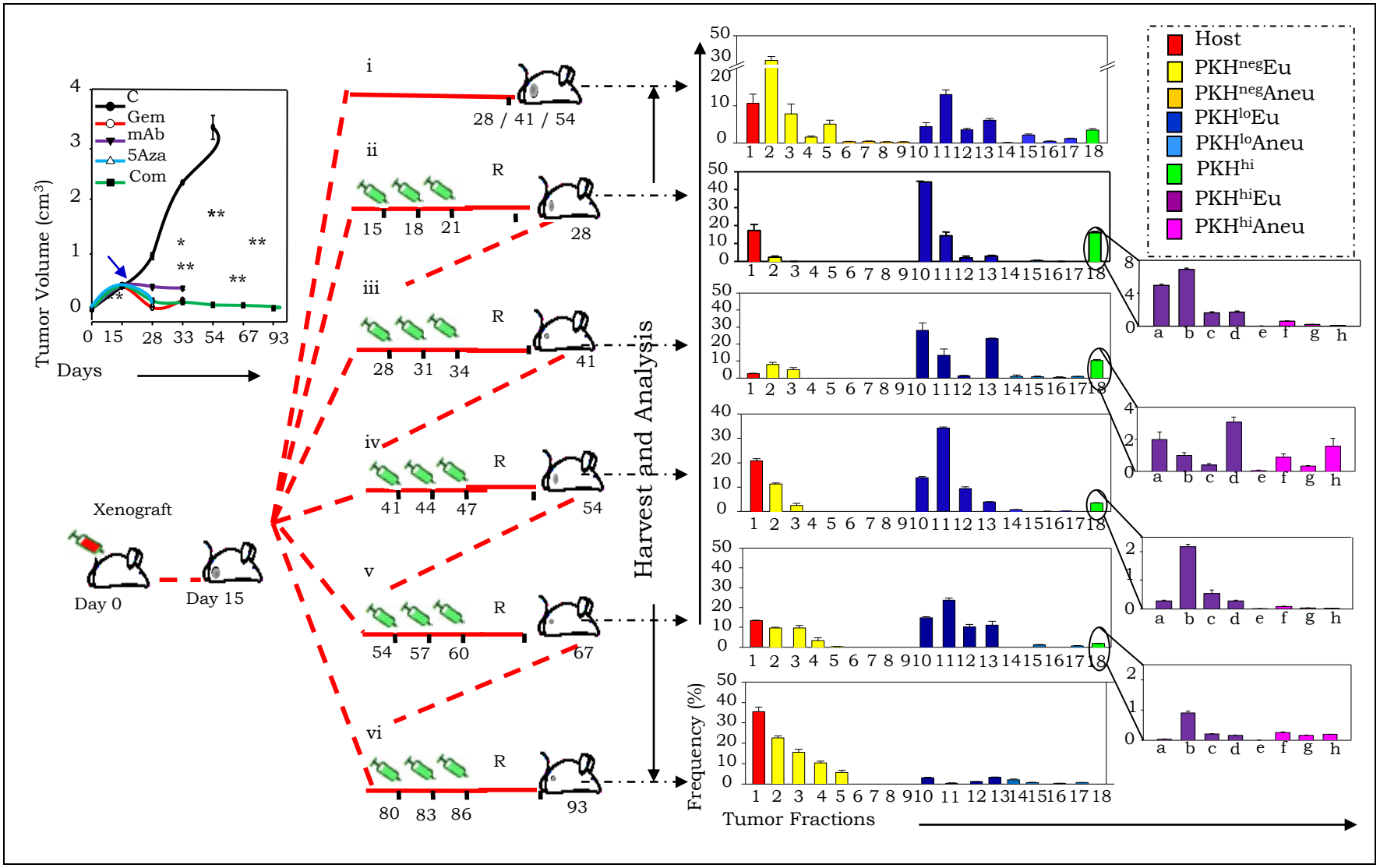
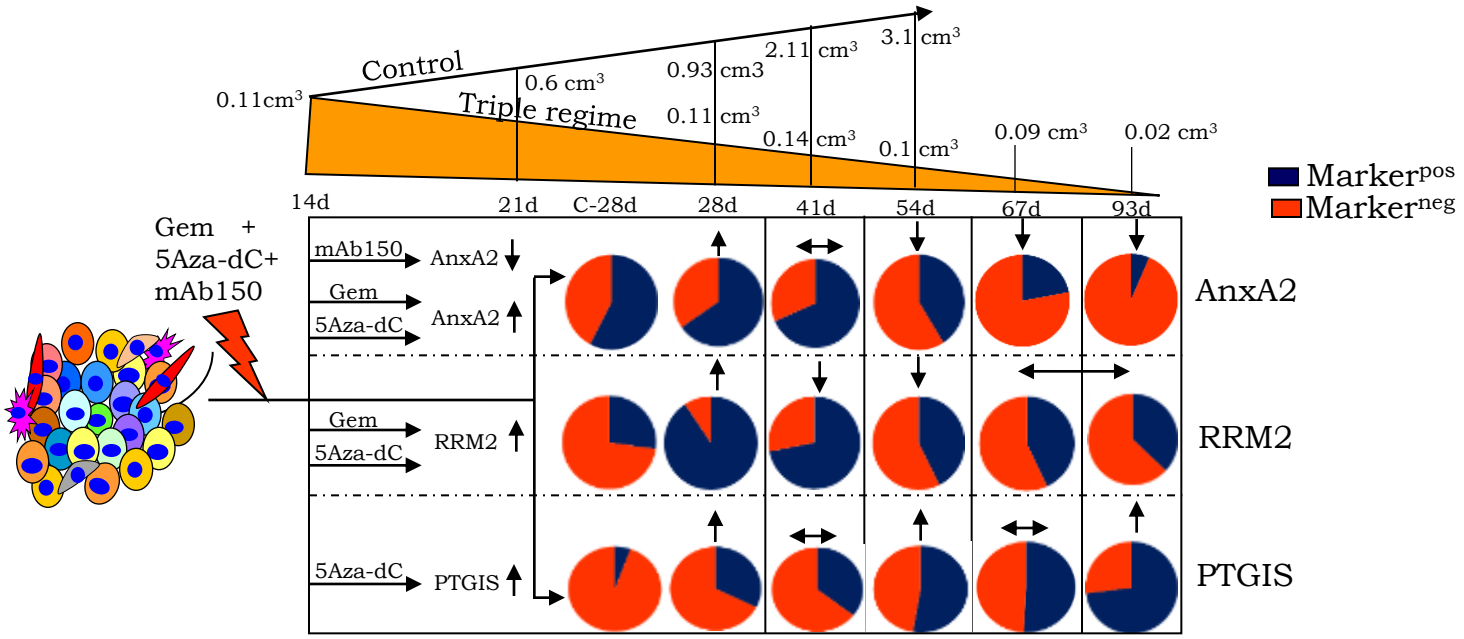
The Cytometry Society 2015 Award for Innovation

Total regression with combinatorial regime Gemcitabine – 5AzadC - mAb150



Combinatorial drug regime : Cell – molecular target cross-talk

(mAb150 – ANXA2; Gemcitabine – RRM2; 5-Aza-dC - PTGIS)



Summary

CSCs – Genetic Instability - Tumor Heterogeneity

Continuous evolution for survival

Changing the Drug Resistance Paradigm

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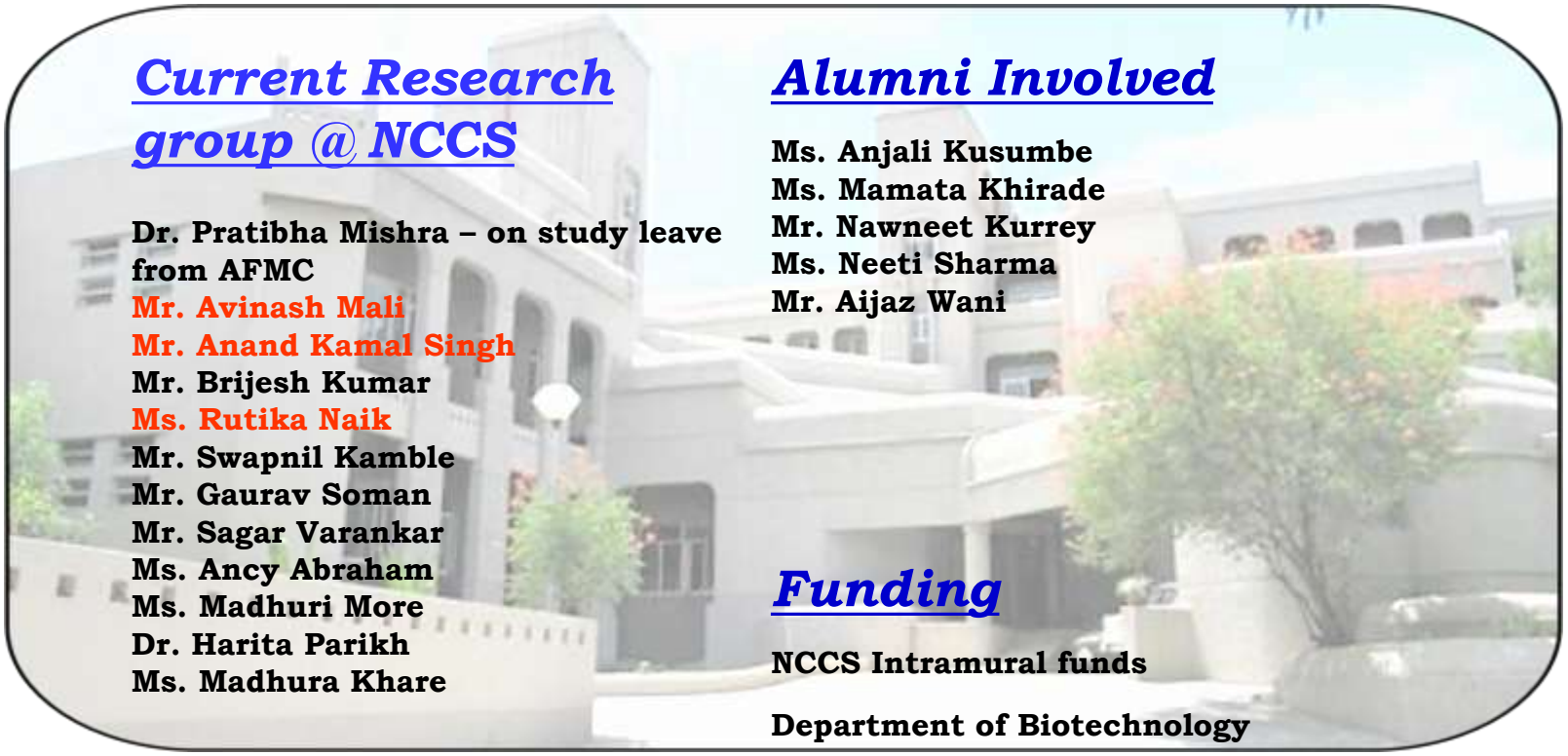
Acknowledgements



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