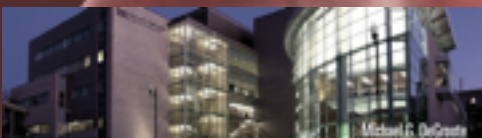


# The Identification of Brain Metastasis Initiating Cells and Regulators of Brain Metastasis From Lung Cancer

**Mohini Singh Hon BSc.**

PhD Candidate, Brain Canada-CIBC Brain Tumor Studentship  
Biochemistry & Biomedical Sciences, Stem Cell & Cancer Research Institute (SCC-RI),  
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Hamilton, ON, Canada



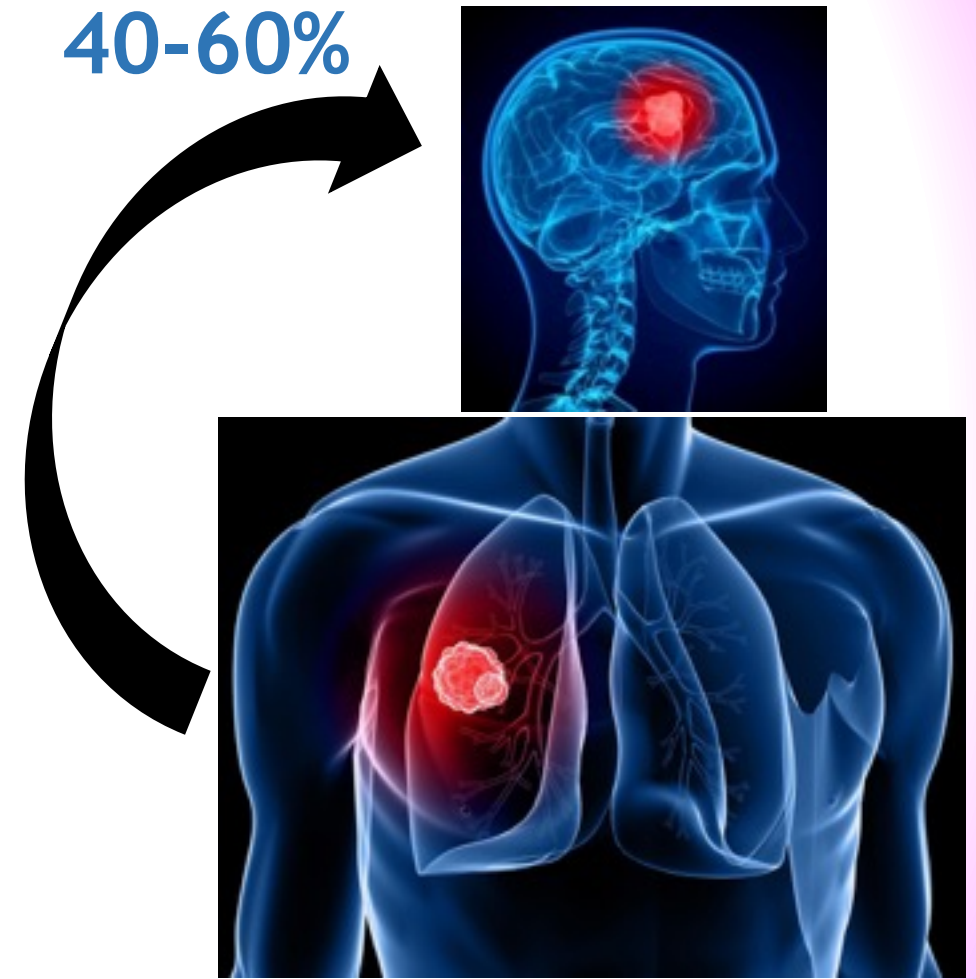
# Lung Cancer and Metastasis to Brain

- Most common cause of cancer-related mortality worldwide
- Causes more deaths than colorectal, breast and prostate cancers combined
- 5 year survival rate is 15%



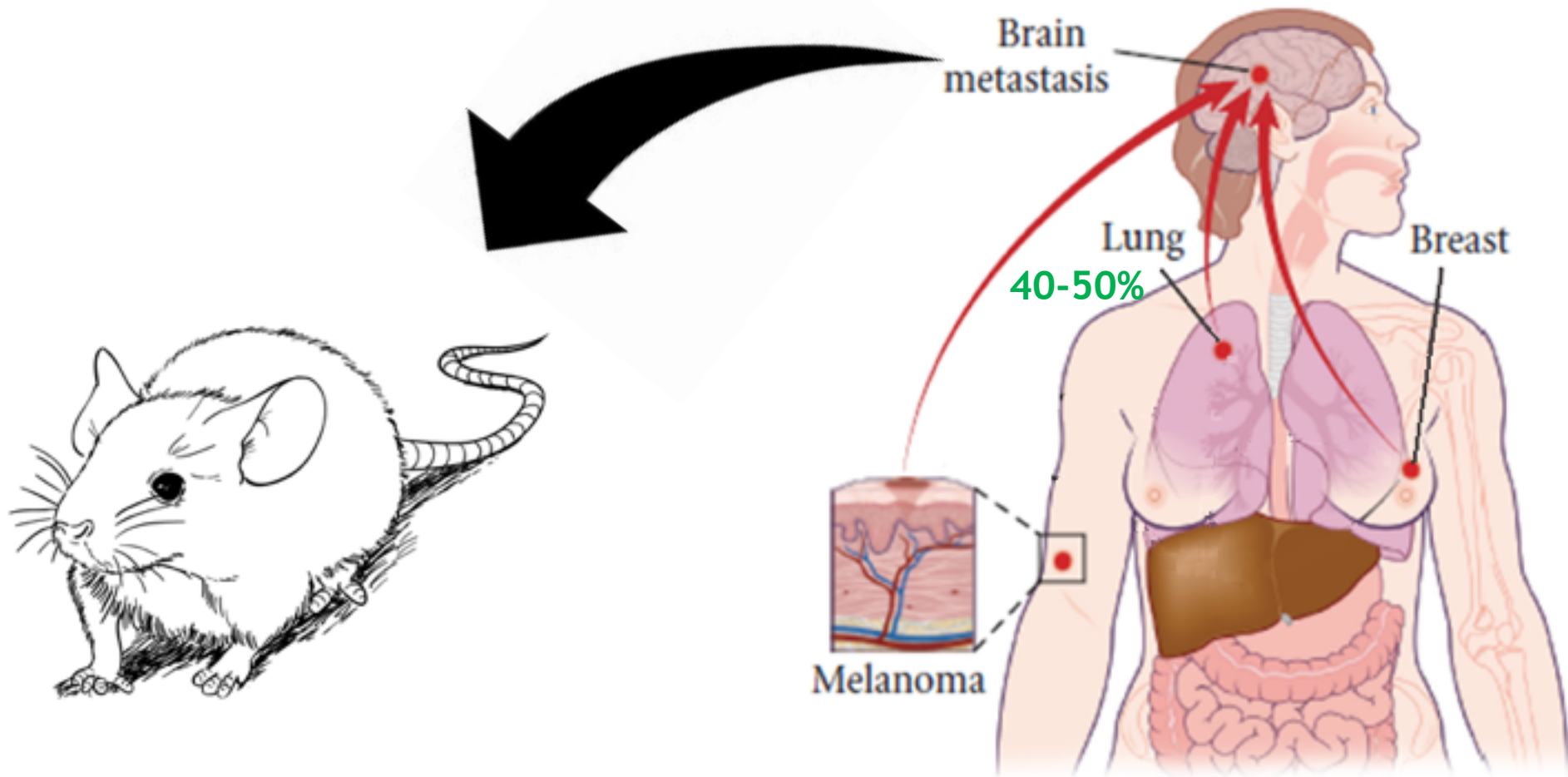
# Lung Cancer and Metastasis to Brain

- Most common cause of cancer-related mortality worldwide
- Causes more deaths than colorectal, breast and prostate cancers combined
- 5 year survival rate is 15%
- Lung cancer is primary site of origin for brain metastasis

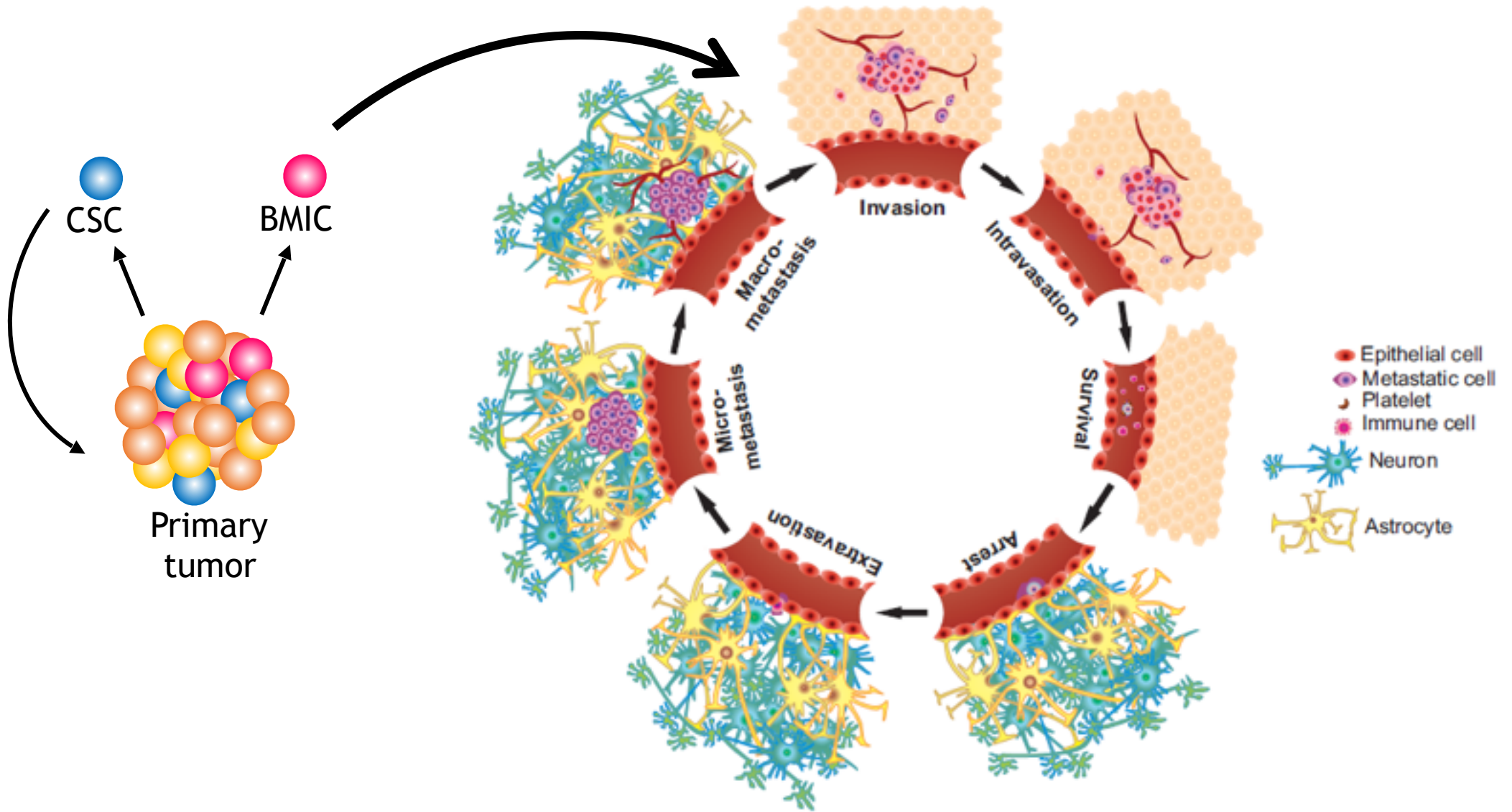




# *In Vivo* Modelling of Metastasis



# Brain Metastasis Initiating Cells (BMIC)

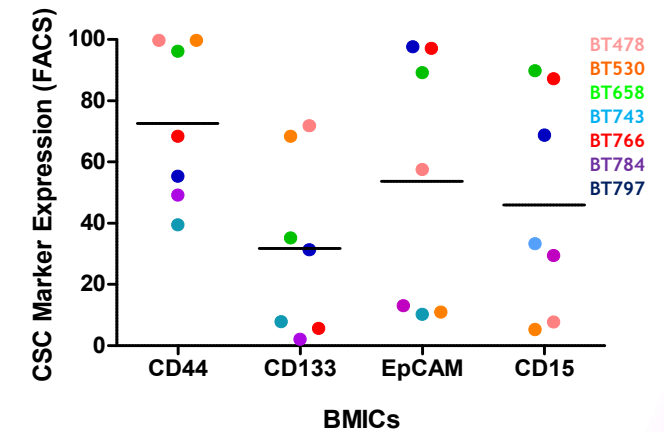
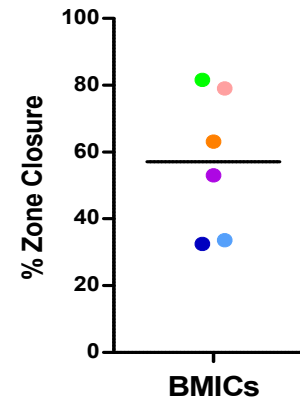
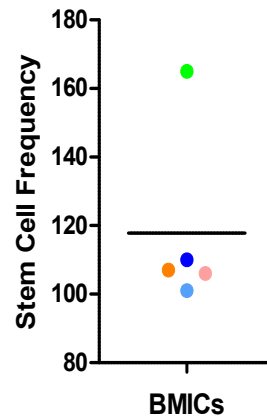
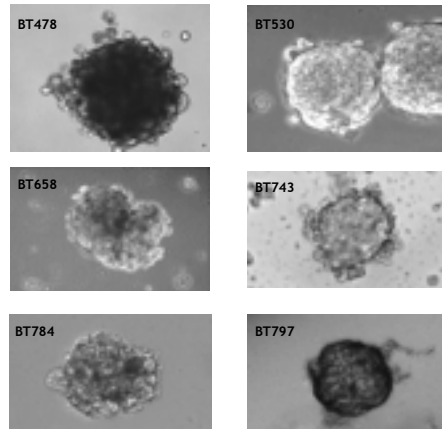


# Lung Derived Brain Metastases Possess CSC-like Cells

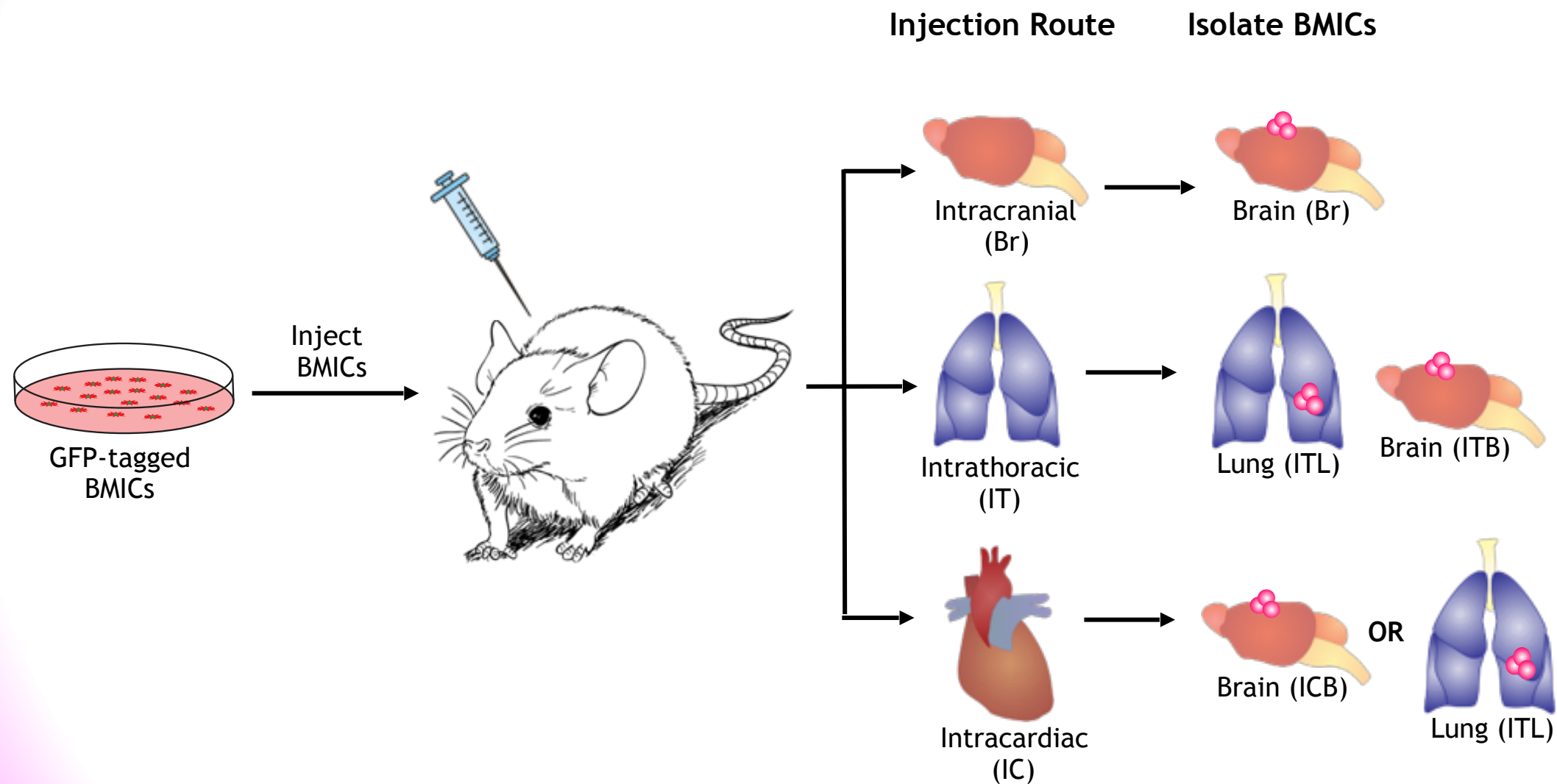
1. **Develop *in vivo* model**
2. **Identify metastatic/tissue specific gene signature**

# Lung Derived Brain Metastases Possess CSC-like Cells

1. Develop *in vivo* model
2. Identify metastatic/tissue specific gene signature

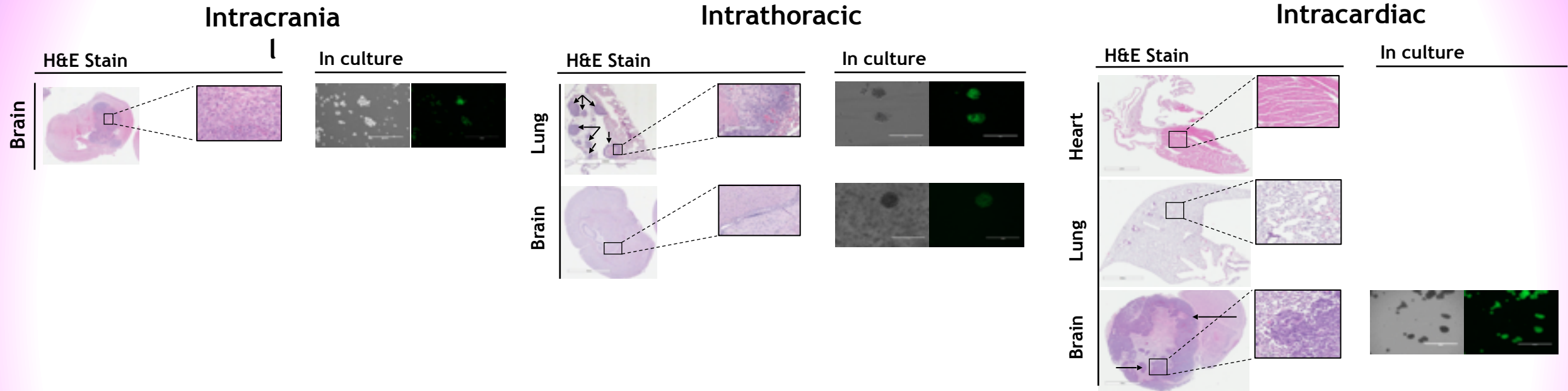


# *In Vivo* Modelling of Brain Metastasis with BMICs

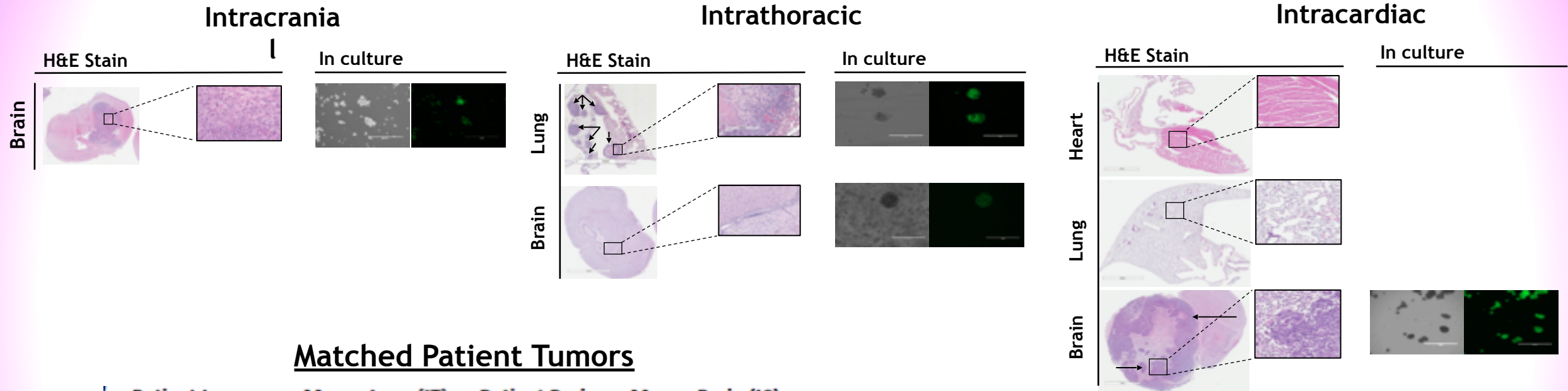




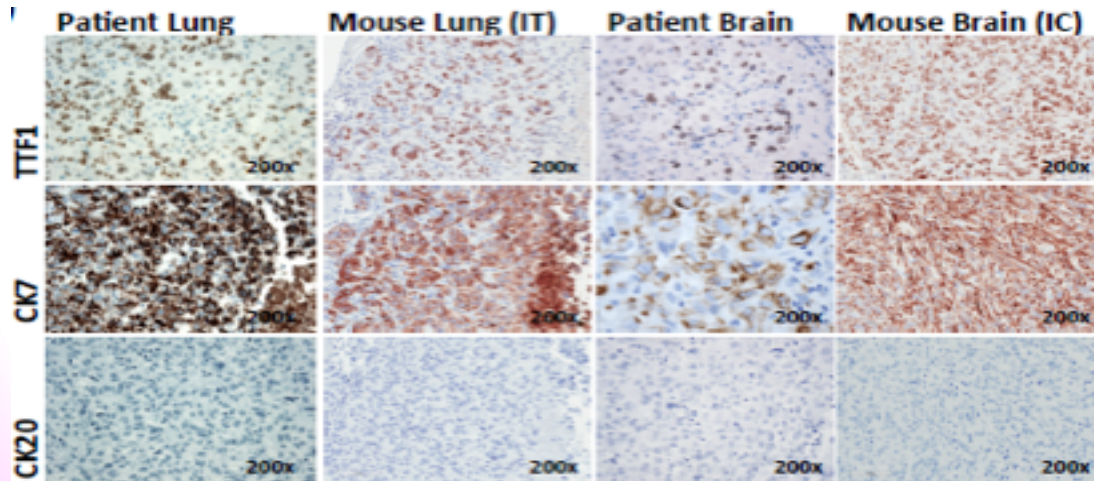
# Isolation of BMICs from *In Vivo* Model



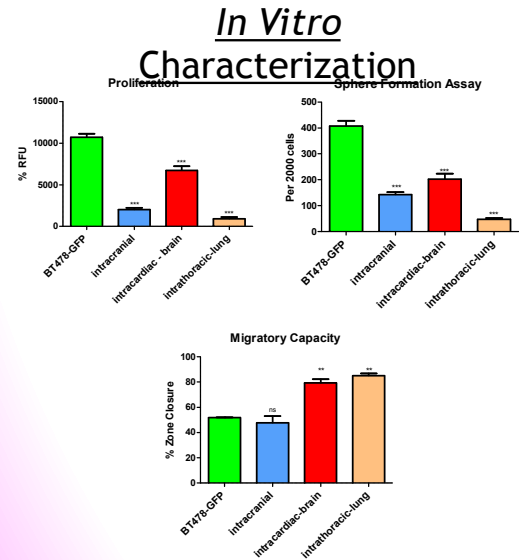
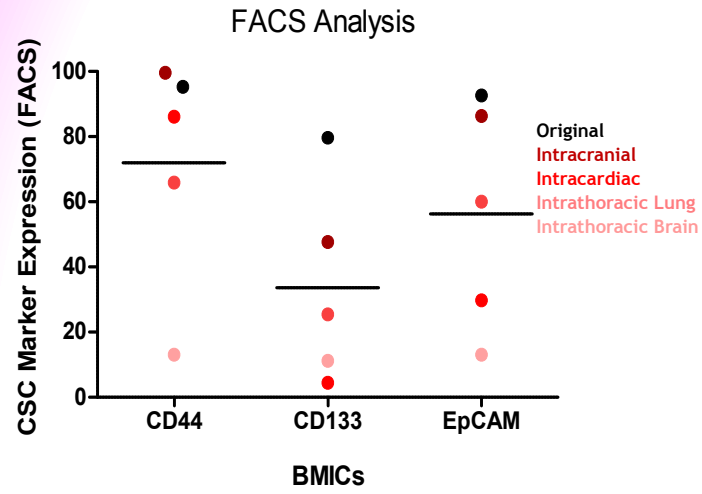
# Isolation of BMICs from *In Vivo* Model



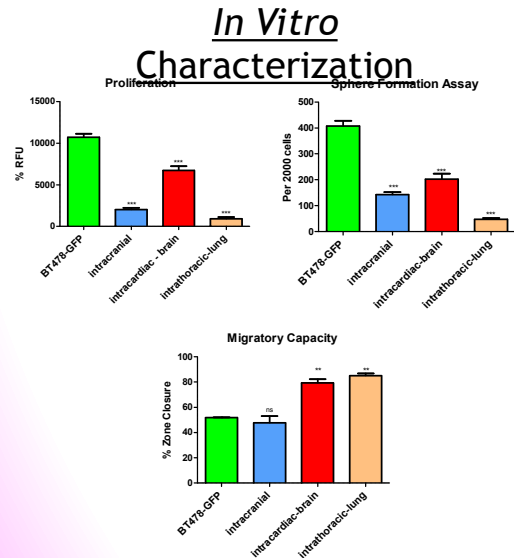
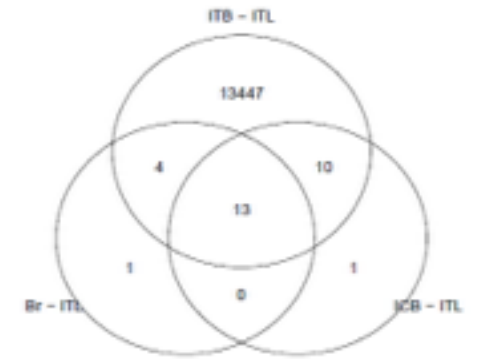
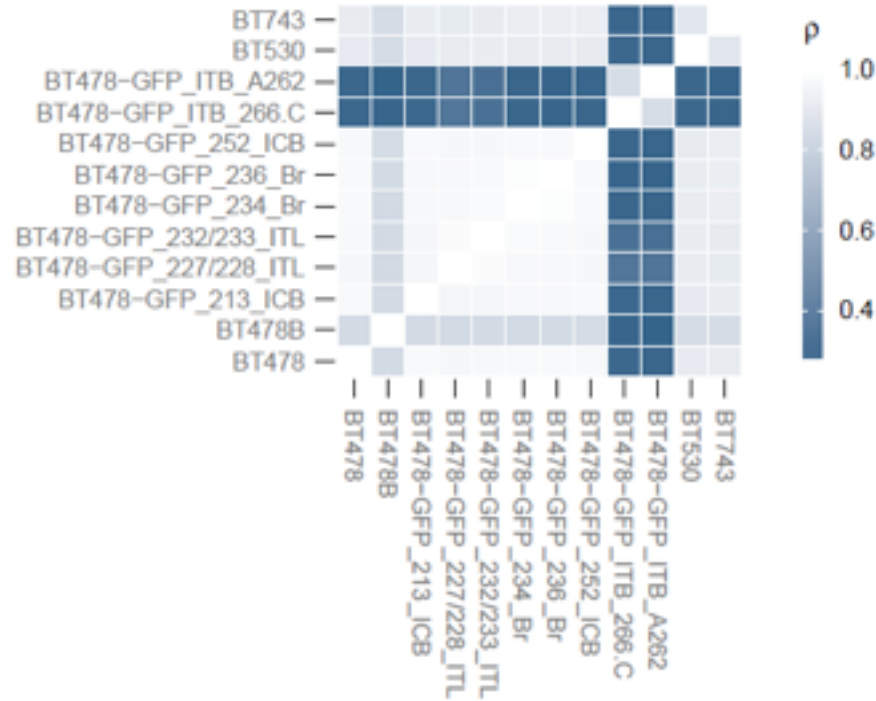
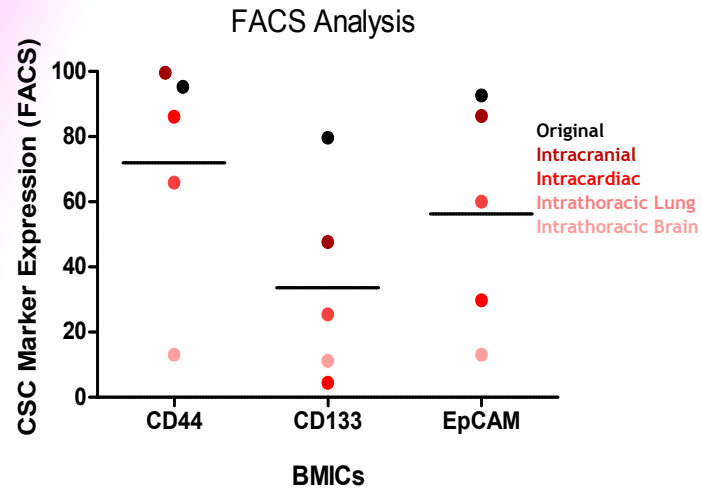
## Matched Patient Tumors



# Characterization of BMICs from *In Vivo* Model

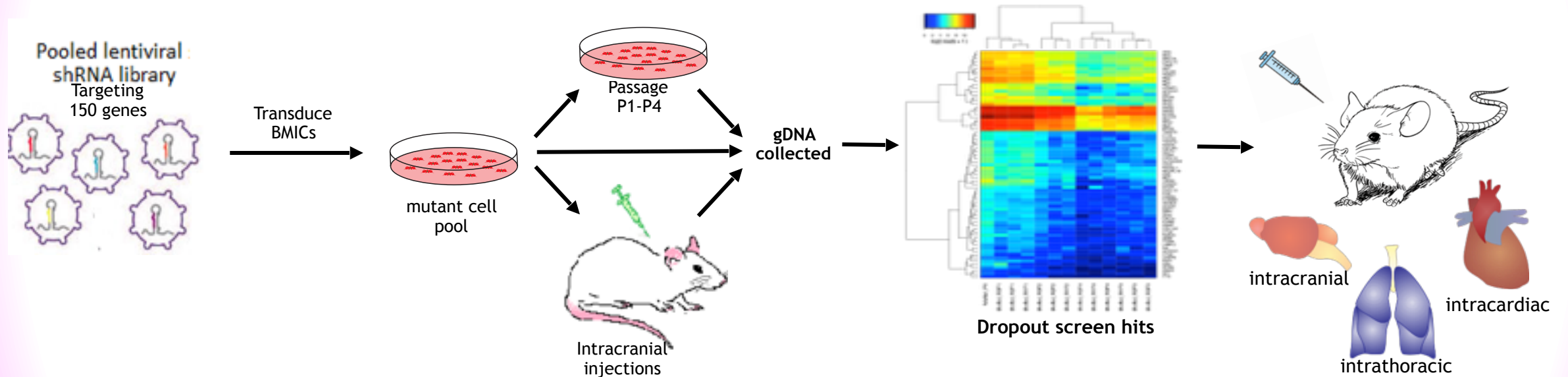


# Characterization of BMICs from *In Vivo* Model

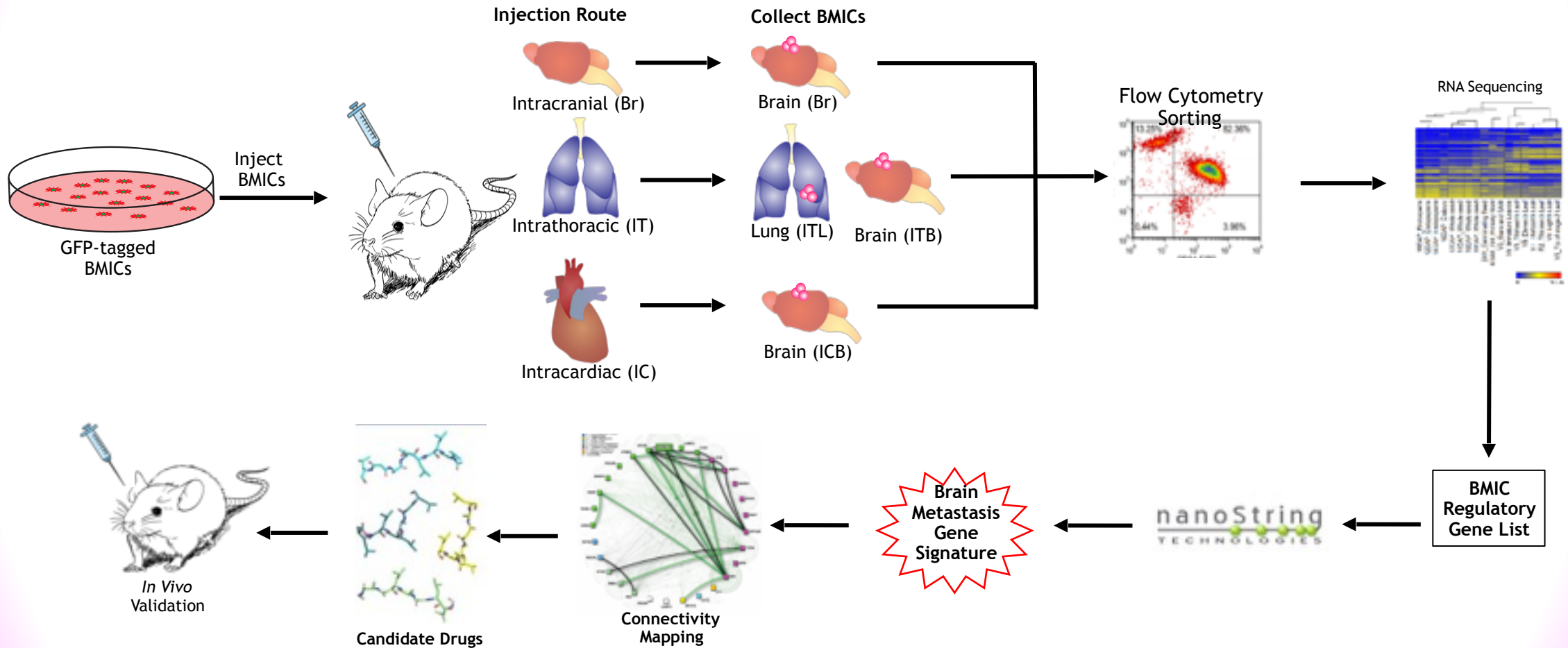




# Validation of Metastatic Genes from shRNA Dropout Screen Using Brain Metastasis Model



# Identification of a BMIC Signature and Clinical Significance



# Acknowledgements

## **Singh Lab Members**

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Sara Nolte

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## **Committee Members**

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Dr. Karun Singh

Dr. Jean-Claude Cutz

## **Everyone in the SCC-RI and Stem Cell Unit**

## **Collaborators**

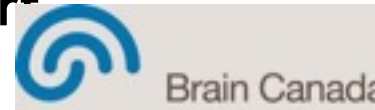
Dr. Jason Moffat & lab

Dr. Igor Jurisica & lab



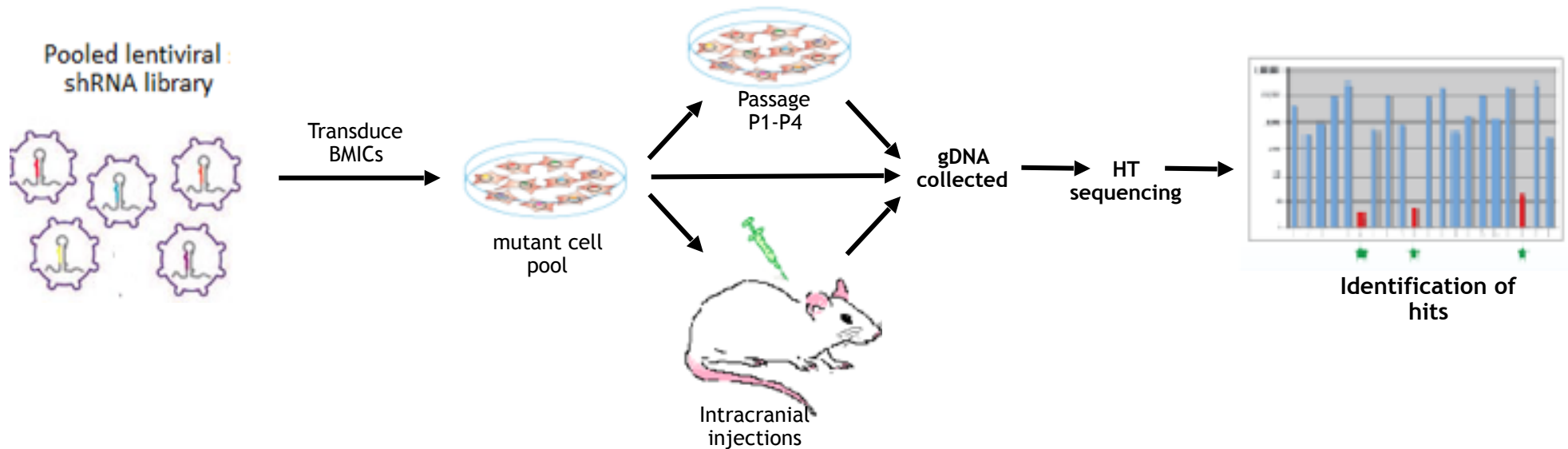
## **Funding Support**

**Boris  
Family**



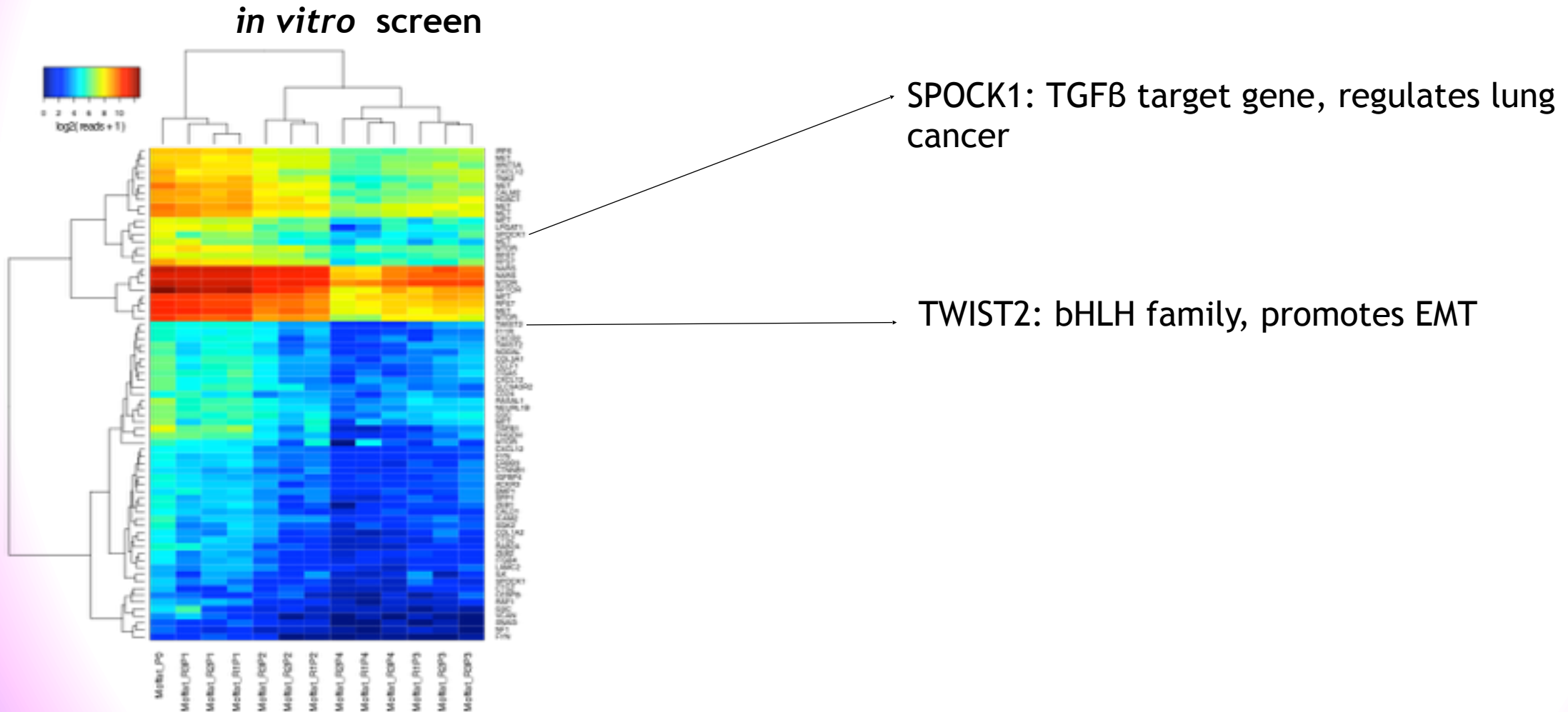
**Société  
canadienne  
du cancer**

# RNAi Screen to validate Genetic Regulators of Metastasis





# Metastatic Gene Signature



# *In Vivo* Validation of Metastatic Genes

# References

- Girouard & Murphy, *Laboratory Investigation* (2011) **91**, 647-664; doi:10.1038/labinvest.2011.50
- Miltenyi Biotec 2015

**Table 3. The advantages and disadvantages of utilizing established cell lines compared with primary cells/tissue to model metastasis**

<b>Tumor Source</b>	<b>Advantages</b>	<b>Disadvantages</b>
Established cell lines	<p>Suitable for high-throughput screening</p> <p>Homogeneous population of cells increases reproducibility</p> <p>Inexpensive, not labor intensive and short time frame (2–3 weeks)</p> <p>Wide variety of cell lines readily available</p> <p>Easy manipulation of gene expression</p>	<p>Low rate of accurate drug responses</p> <p>Poor ability to retain original molecular profile in vitro</p> <p>Interspecies tumor-stroma compatibility</p> <p>Homogeneous population of cells less closely mimics human metastasis</p>
Primary tissue/cells	<p>Heterogeneous population of cells more closely mimics human metastasis</p> <p>Human stromal responses are partially accounted for</p> <p>Potential application to develop personalized therapy</p> <p>Better maintenance of original molecular signature and cancer stem cells</p>	<p>Low rate of phenotypic reproducibility owing to heterogeneity</p> <p>Long latency, expensive and labor intensive</p> <p>Not suitable for high-throughput screening</p> <p>Gene manipulation is challenging</p> <p>Limited availability of fresh tissue</p>



# Metastatic gene signature

**Table 1 • The 17-gene signature associated with metastasis**

Gene	Gene name	GenBank ID
Upregulated in metastases		
<i>SNRPF</i>	Small nuclear ribonucleoprotein F	AI032612
<i>EIF4EL3</i>	Elongation initiation factor 4E-like 3	AF038957
<i>HNRPAB</i>	Heterogeneous nuclear ribonucleoprotein A/B	M65028
<i>DHPS</i>	Deoxyhypusine synthase	U79262
<i>PTTG1</i>	Securin	AA203476
<i>COL1A1</i>	Type 1 collagen, $\alpha$ 1	Y15915
<i>COL1A2</i>	Type 1 collagen, $\alpha$ 2	J03464
<i>LMNB1</i>	Lamin B1	L37747
Downregulated in metastases		
<i>ACTG2</i>	Actin, $\gamma$ 2	D00654
<i>MYLK</i>	Myosin light chain kinase	U48959
<i>MYH11</i>	Myosin, heavy chain 11	AF001548
<i>CNN1</i>	Calponin 1	D17408
<i>HLA-DPB1</i>	MHC Class II, DP $\beta$ 1	M83664
<i>RUNX1</i>	Runt-related transcription factor 1	D43969
<i>MT3</i>	Metallothionein 3	S72043
<i>NR4A1</i>	Nuclear hormone receptor TR3	L13740
<i>RBMS</i>	RNA binding motif 5	AF091263

Ramaswamy

the genetic state of the primary tumor reflects the ability of that cancer to metastasize

Lee 2012

chromosomal copy number alterations (CNA) of those FFPE samples were analysed by the Molecular Inversion Probe (MIP) technology, the most frequent CNAs detected. Those regions harbour several candidate genes including NeurL1B, ACTA2, FAS and ICAM2.

13-gene signature to predict rapid development of brain metastases in patients with HER2-positive advanced breast cancer.

[Renata Duchnowska](#)

Publication	Array Platform	Samples description	Gene signatures	Matching
Yan's Year et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature, 2002.	Agilent 24479 60-mer oligos	97 samples from 26 patients	220 prognosis reporters (risk of distant metastasis)	0 (bone, brain, liver and lung)
			400 Breast reporters	0 (bone, brain, and lung) HMGCS1 (liver)
			1400 ER-reporters	AP1, P1, UCP1, PTPN3 (bone) 0 (brain,liver) DSC2, UGT8 (lung)
Ramseyer et al. A molecular signature of metastasis in primary solid tumors. Nat Genet, 2005.	Rosetta Inkjet (24479 genes; breast adenocarcinoma oligonucleotide microarrays.	279 primary tumors of diverse types (lung, breast, prostate)	128 genes separating metastasis from primary tumors able to distinguish patients with good versus poor prognosis	0 (bone, brain, liver and lung)
Harris et al. Towards integrated clinical-genomic models for personalized medicine: combining gene expression signatures and clinical factors in breast cancer outcomes prediction. Human mol. genetics, 2005.	Multiple gene expression signatures "metagenes"	86 LIn breast cancer patients	142 predictors of lymph node metastasis	PTNRC (bone) SQLE (brain) 0 (liver and lung)
			165 predictors of breast cancer recurrence	CSF1 (bone) UGT1 (brain) 0 (liver) PGR (lung)
Wang et al. Gene expression profiles to predict distant metastasis of lymph-node negative primary breast cancer. The Lancet, 2005.	Affymetrix U133A 25-mer oligos	LIn and LIn+ patients with invasive breast cancer	76-gene signature to distinguish LIn- primary breast cancer to develop distant metastasis within 5 years	0 (bone, brain, liver and lung)
Nadiri et al. A gene-expression signature to predict survival in breast cancer across independent data sets. Oncogene, 2006.	Agilent 22575 60-mer oligos	135 tumor samples (no criteria for selection)	70-gen prognostic signature (risk of metastasis)	0 (bone, brain, liver and lung)
Huyan et al. Predicting a local recurrence after breast-conserving therapy by gene expression profiling. Breast Cancer Res, 2006.	Agilent 24479 60-mer oligos	167 patients in stage I and II breast cancer with age < 52 years	70-gene predictor of local recurrence	0 (bone, brain, liver and lung)
Feng et al. Differentially expressed genes between primary cancer and paired lymph node metastases predict clinical outcome of node-positive breast cancer patients. Breast Cancer Res Treat, 2006.	Q-scan 70-mer two-color 11239 probes	35 patients: primary tumor and lymph node metastasis paired samples	79 differentially expressed genes between primary samples and metastasis samples	PTNRC (bone) LUN (brain) 0 (liver) PGR (lung)
Wen et al. Genes that mediate breast cancer metastasis to lung. Nature, 2005	Affymetrix U133A	82 breast cancer patients (Primary tumors)	95 genes predictors of lung metastasis	CSF1 (bone) 0 (brain, liver, lung)

(CXCL12, DSC2 and TFDP2) were associated with progression to specific organs when tested in an independent dataset

Klein et al. Identification of brain- and bone-specific breast cancer metastasis genes. Cancer Letters, 2006	Affymetrix U133A	8 bone metastases, 18 brain metastases and 3 primary tumours	51 brain metastasis specific genes (vs. bone metastasis)	0 (bone, brain, liver) UGTS (lung)
Box et al. Genes that mediate breast cancer metastasis to the brain. Nature, 2009	Affymetrix U133A	CH34-B440 and MDA231-B162 brain metastatic cell lines. 368 breast cancer primary tumors	17 genes whose expression was correlated with brain relapse	0 (bone, brain, liver, lung)
			26 genes whose expression was increased in brain metastatic cell lines but not in bone or lung metastatic cell lines	0 (bone, brain, liver, lung)

# CD133 CD44EpCAM

- EpCAM: epithelial cell adhesion molecule, transmembrane glycoprotein that mediates calcium independent cell cell adhesion in epithelia. Also involved in proliferation, migration, differentiation, and metastasis - upon cleavage forms a complex with bet-catenin etc to promote translation of several oncogenes. Marker of neoplasms of epithelial origin
- CD133: 5 transmembrane glycoprotein. Function unknown, but used as a marker for CSCs of various cancers ie. GBM and colorectal
- CD44: cell surface glycoprotein involved in cell cell interactions, cell adhesion and migration. Receptor for hyaluronan, and marker for CSCs from breast and prostate. CD44 is a regulator of several miRNAs known to maintain CSCs. It has multiple isoforms, including CD44H, which exhibits high affinity for hyaluronate, and CD44v6 which has metastatic properties.

# In Vivo Modelling of Metastasis

## Limiting Dilution Assay

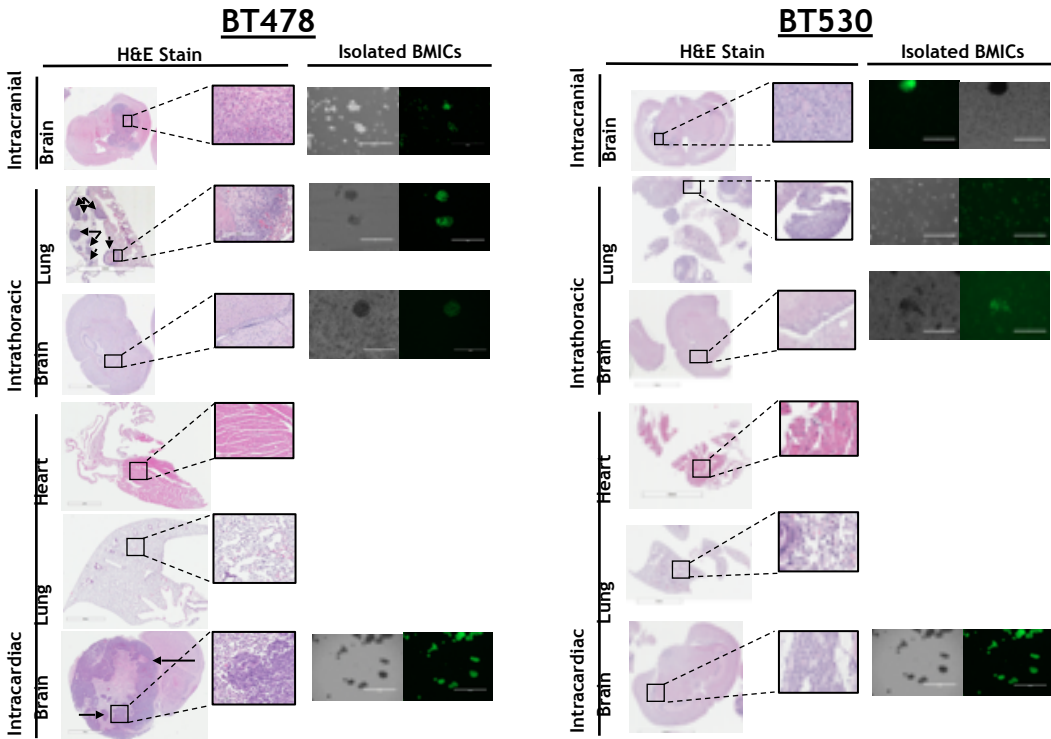
BT478

intrathoracic	# cells	mice injected	frequency of tumours
	1,000,000	8	0
	500,000	32	12
	250,000	2	0
	100,000	2	0
intracranial	# cells	mice injected	frequency of tumours
	100,000	2	2
	50,000	3	3
	1,000	2	2
	100	4	4
intracardiac	# cells	mice injected	frequency of tumours
	1,000,000	2	2
	250,000	16	11
	200,000	5	0
serial	250,000	4	3

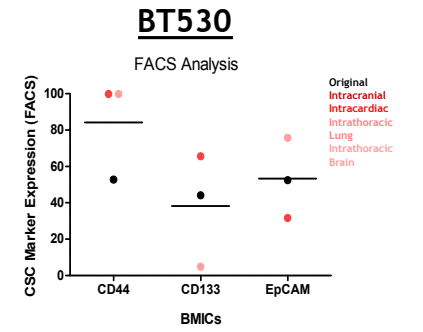
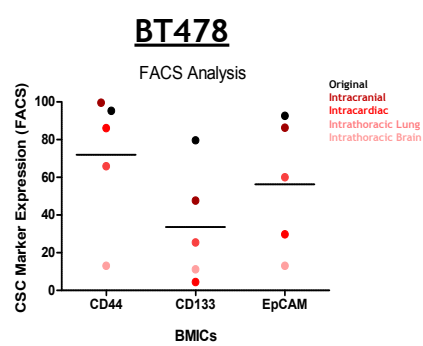
BT530

intrathoracic	# cells	mice injected	frequency of tumours
	500,000	4	4
intracranial	# cells	mice injected	frequency of tumours
	100,000	2	2
	50,000	3	3
	100	2	2
intracardiac	# cells	mice injected	frequency of tumours
	250,000	4	2

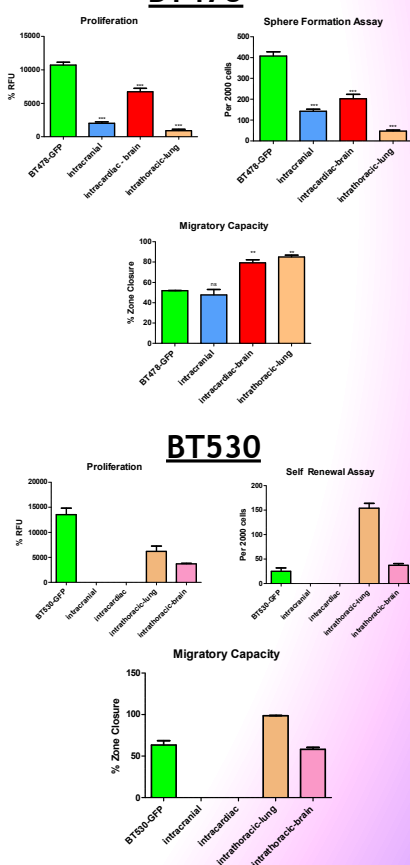
## Isolated BMICs



## FACs Characterization

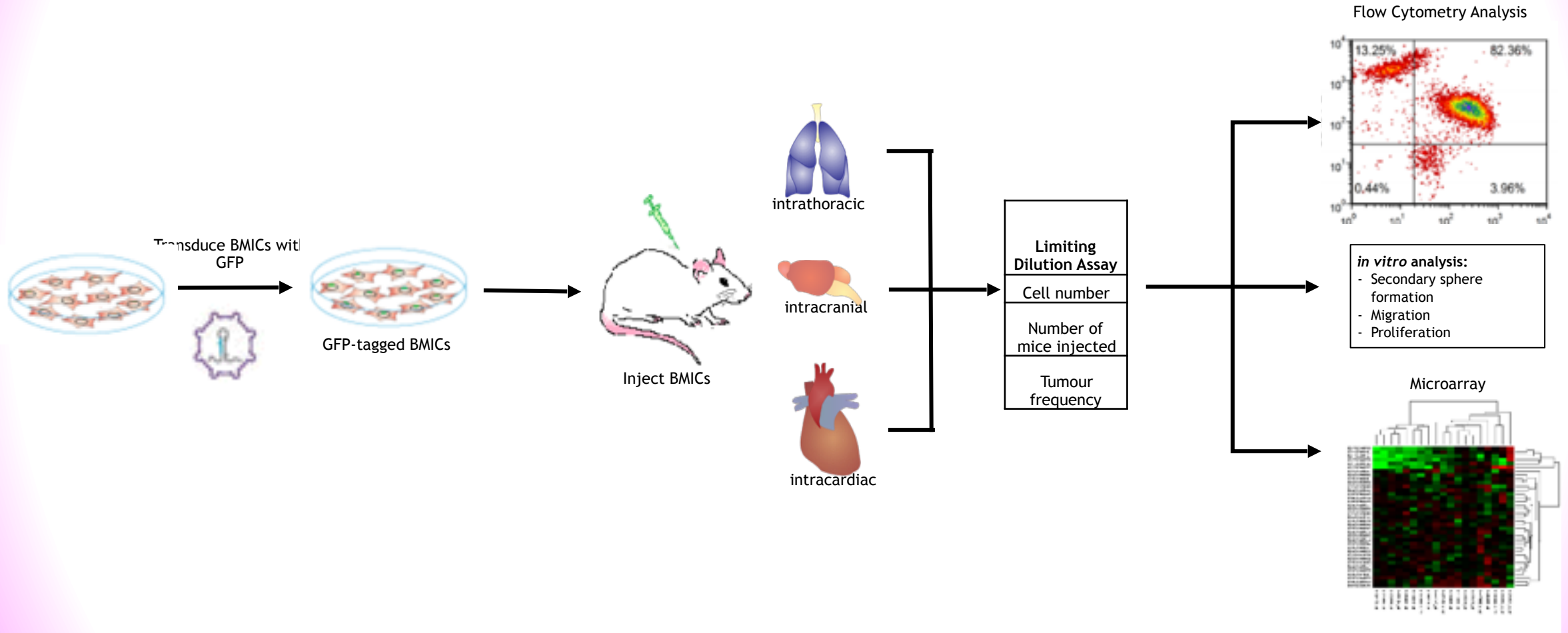


## In Vitro Characterization



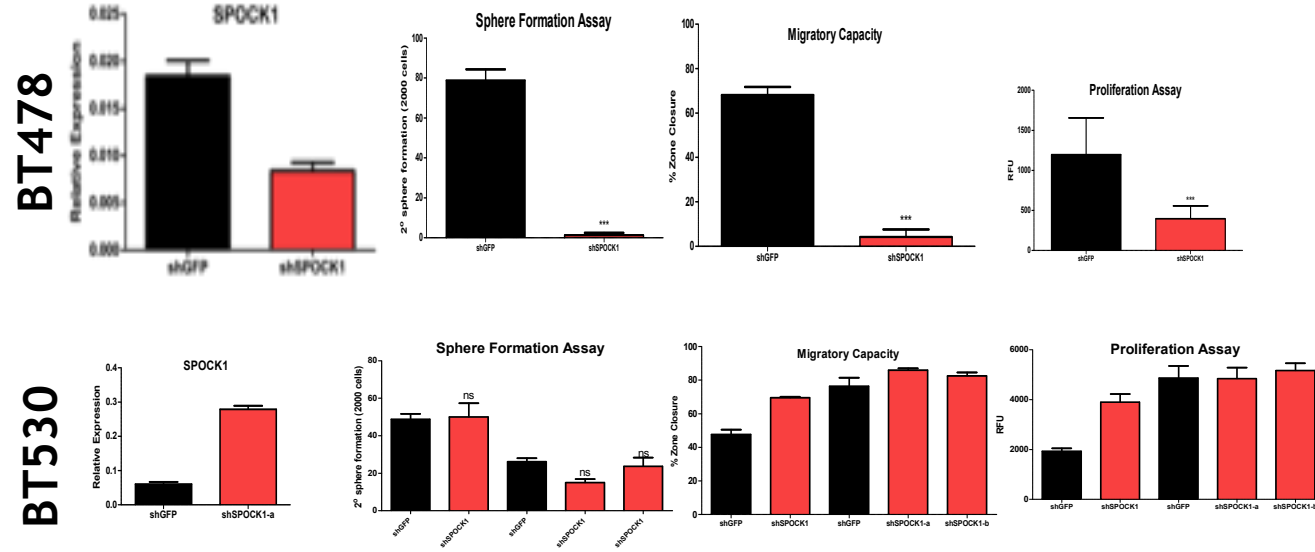


# *In Vivo* Modelling of Metastasis



# *In Vitro* Validation of Metastatic Genes

SPOCK1



TWIST2

