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***Characterization of Autologous
Smooth Muscle Cells Following
Exposure to Bladder Cancer Drugs***

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Confidential

Study Objective

Objective: Study the phenotypic and genetic stability of SMC during ex vivo expansion after exposure to neoadjuvant chemotherapy drugs

- *Model the impact of chemotherapy regimens on adipose-derived SMC (Ad-SMC) by exposure of healthy cell cultures to cancer drug combinations*
- *Compare characterization profile to bladder SMC (BI-SMC) obtained from bladder cancer patients*

Study Design and SMC Characterization Plan

Samples

Adipose-derived SMC
Tissue samples from two healthy females
(Ages: 25, 44 yrs)

Bladder-derived SMC
Cadavers & Bladder Cancer Patients
(Ages: 52 to 81 yrs)



Growth Medium
 \pm
Chemotherapy Drugs at p1

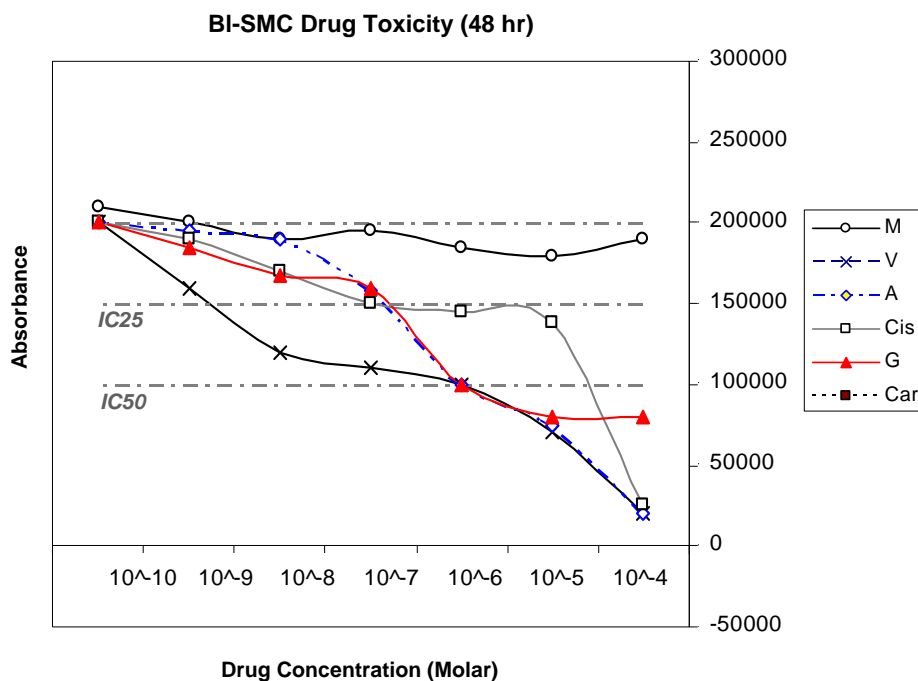
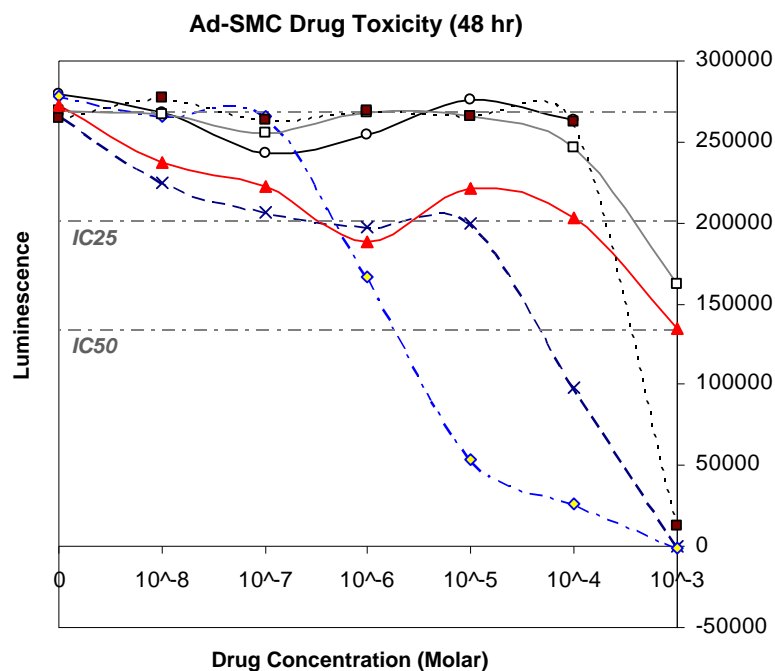


Recovery and Cell Expansion
&
Production of Study Samples
(p1 to p4)

SMC Characterization

- **Cell Morphology**
- **Cell Phenotype (SMC α -actin)**
- **Ploidy (Cell Cycle / DNA Content)**
- **Karyotype (Chromosome Assessment)**

In vitro Drug Dose Determination



- **A High (IC₅₀) and a Low (IC₂₅) inhibitory concentration for cancer drugs was established in vitro**
- **In-vitro inhibitory drug concentrations are 100-1000 fold higher than therapeutically used in the clinic***
- **Ad-SMC shows a reduced sensitivity to cancer drugs in comparison to BI-SMC**

* CCO Formulary (Cancer Care of Ontario, 2010)

Testing Plan

– Ad-SMC & BI-SMC

Adipose derived SMC were exposed to cancer drugs in vitro

Ad-SMC - <i>in vitro</i> Drug Treatment			
Groups	Tissue	Drugs ¹	Exposure (24 & 48 hr)
Control	1	None	
	2	None	
Group 1 (C:Cisplatin)	1	GC	High
			Low
		MVAC	High
			Low
	2	GC	High
			Low
		MVAC	High
			Low
Group 2 (C:Carboplatin)	1	GC	High
		MVAC	Low
	2	GC	High
		MVAC	Low

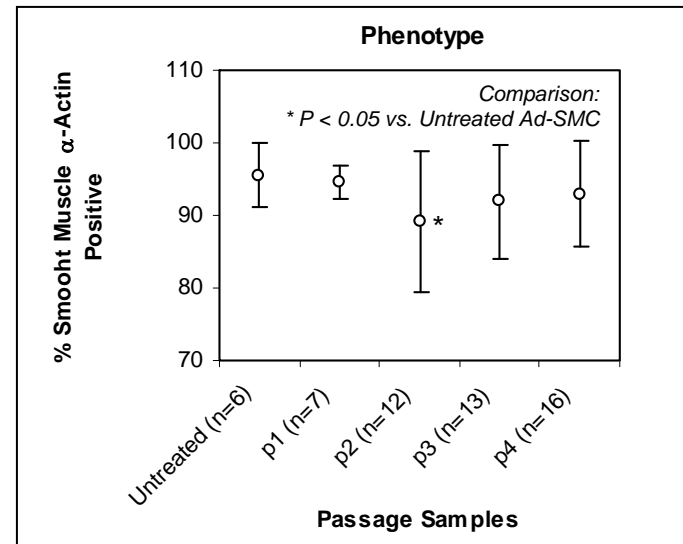
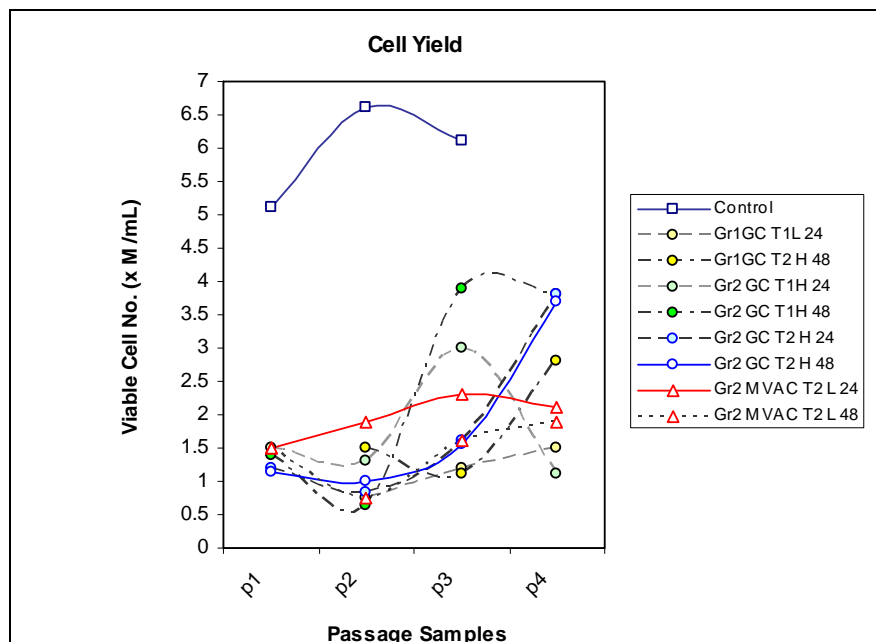
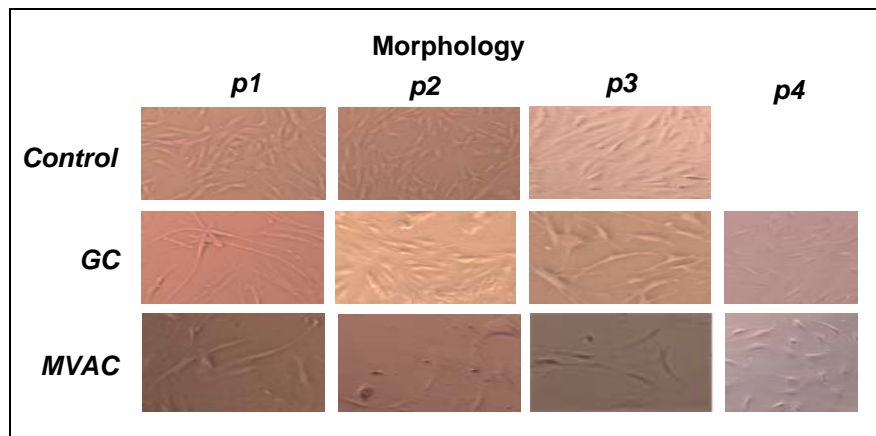
BI-SMC - Clinical Drug Treatment		
Groups	Prior Chemo / Radiation Clinical Treatment	<i>in vitro</i> Chemotherapy Exposure
Treated	Yes	No
Untreated	No	

Representative samples in data shown

¹ GC: Gemcitabine + Platinum compound (Cisplatin or Carboplatin);
 MVAC: Methotrexate + Vinblastine + Adriamycin + Platinum compound.

SMC Characterization

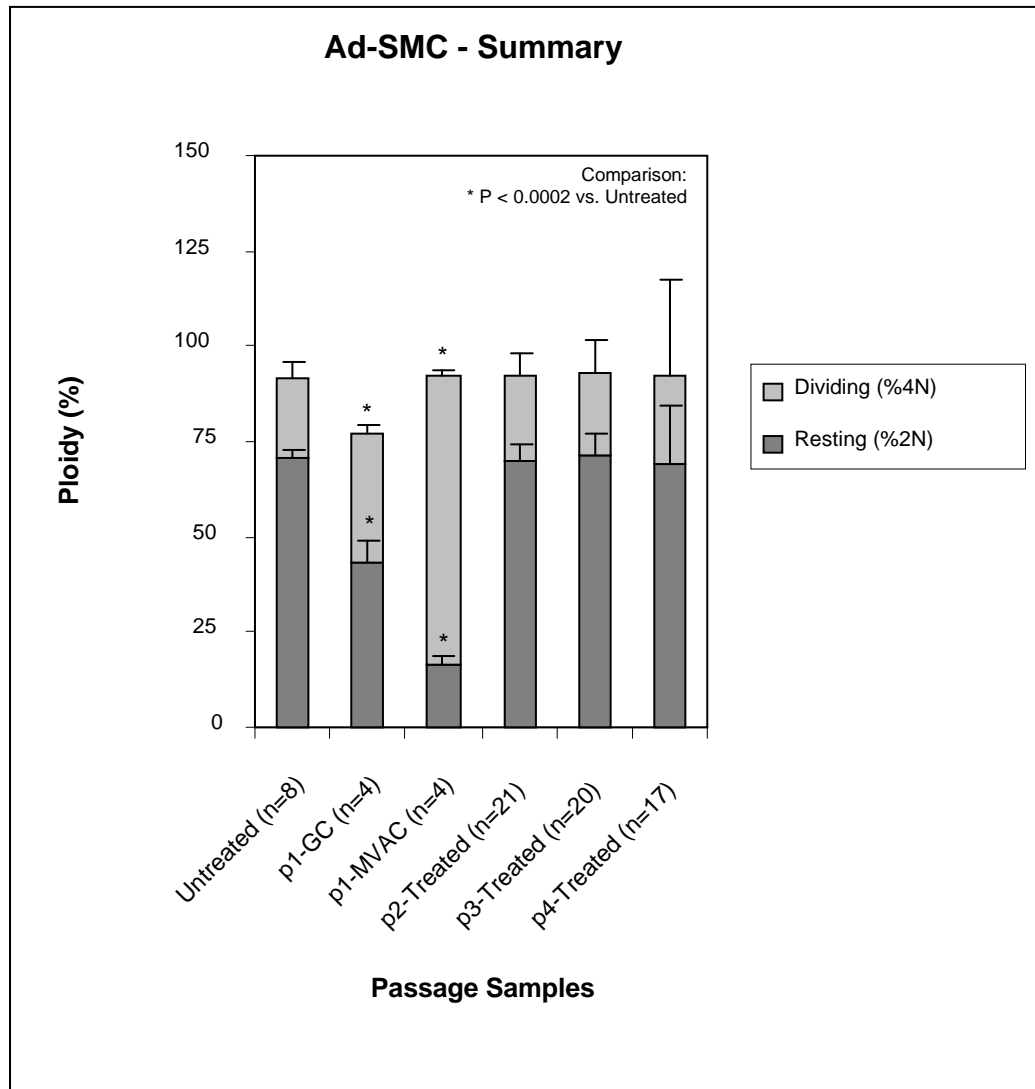
- Morphology, Phenotype and Cell Yield



- **SMC morphology & phenotype is retained following drug withdrawal**
- **Proliferative potential recovers after serial passages**

SMC Characterization

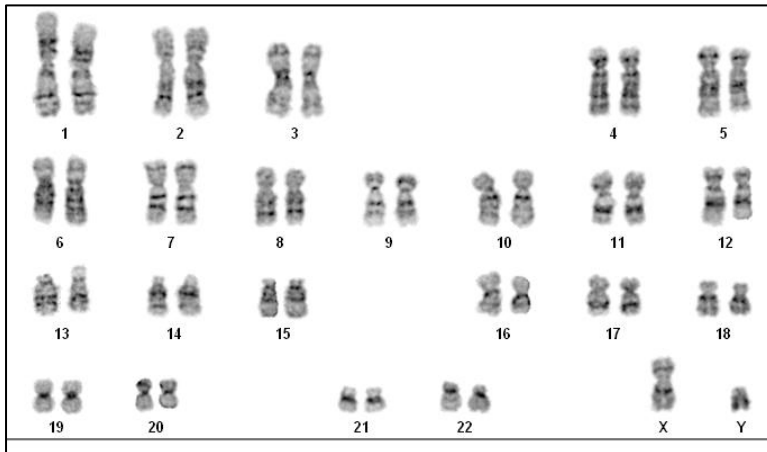
- Ploidy



➤ *Ploidy recovers within short-term culture following drug withdrawal*

SMC Characterization

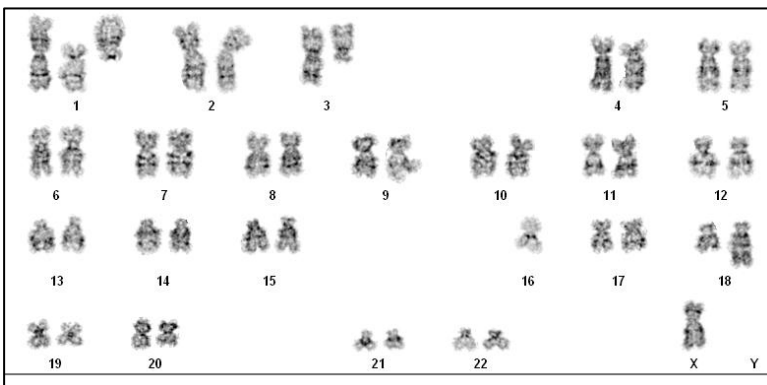
Karyotype – Sample Analysis



46,XY [20]

Sample Calculations

Karyotype	46,XY		Altered		Total Alterations
	Number	Ratio	Number	Ratio	
46,XY [20]	20 of 20	1.0	0 of 20	0	0
46,X,-Y,del(1)(p21),+del(1)(p10),t(3;18)(q12;q23) [1] /45,X,-Y,inv(7)(p15;q34) [8] /45,X-Y [10] /46,XY [1]	1 of 20	0.05	19 of 20	0.95	34



45,X,-Y,del(1)(p21),+del(1)(p10),t(3;18)(q12;q23, -16)

➤ **Analysis enables quantitative assessment of chromosome quality**

SMC Characterization

Survey of Chromosomal Alterations in Healthy Individuals

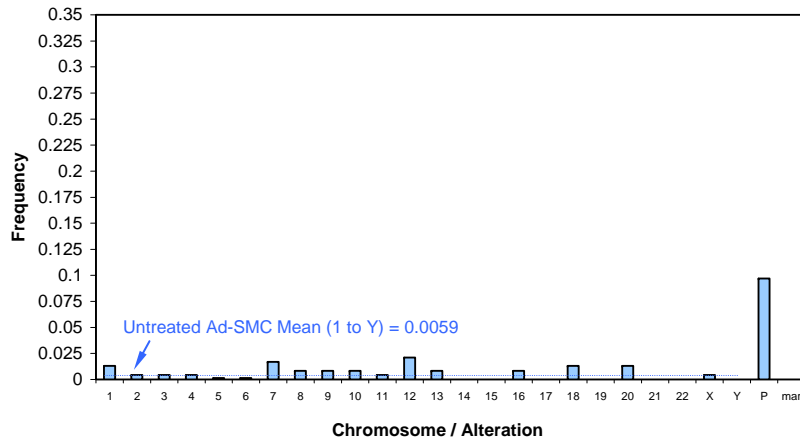
Prevalence of Chromosomal Alterations in Healthy Subjects		
Frequency	Type of Alterations	References
0 to 2%	Mixed (structural & numerical changes)	Lopez de Mesa <i>et al.</i> (2000)
4 to 7%	Monosomies (single chromosomes)	Gebhart <i>et al.</i> (1993), Kinne <i>et al.</i> (2001)
0.5 to 20%	Polyploidy in SMC (multiple chromosomes)	Storchova <i>et al.</i> (2008)
25%	Loss of X or Y chromosome	Wong <i>et al.</i> (2008)
Variable	Mixed, residual chemotherapy treatment effect	Rosier <i>et al.</i> (2003), Castro Kreder <i>et al.</i> (2004)
Variable	Fragile Site Instability	Glover <i>et al.</i> (2005), Lukusa & Fryns (2008), Mrasek <i>et al.</i> (2010)
Variable	Copy Number Variants	Fanciulli <i>et al.</i> 2009), Wain <i>et al.</i> (2009)
Variable	Tolerance to Aneuploidy	Swami (2010), Cetin & Cleveland (2010)

➤ ***Karyotype alterations are common and occur at variable frequencies in healthy subjects***

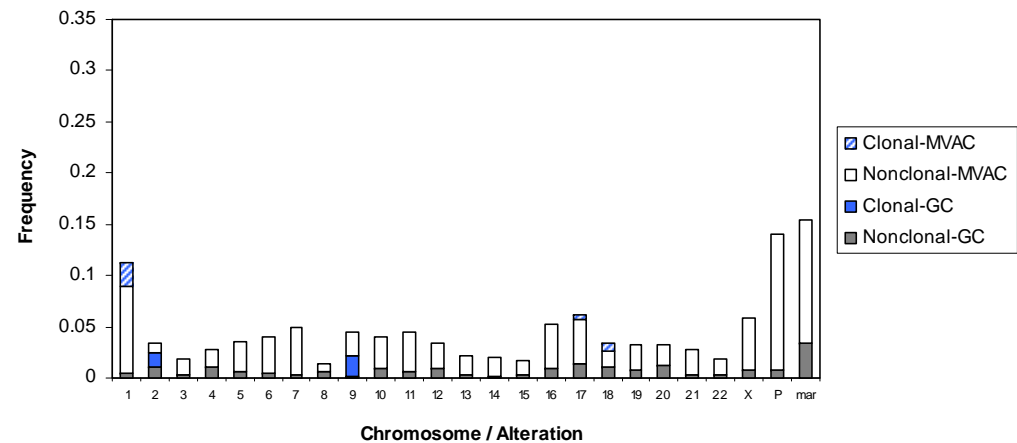
SMC Characterization

Karyotype – Frequency of Alterations

Ad-SMC - Untreated



Ad-SMC - Treated
(Combined T1 & T2)

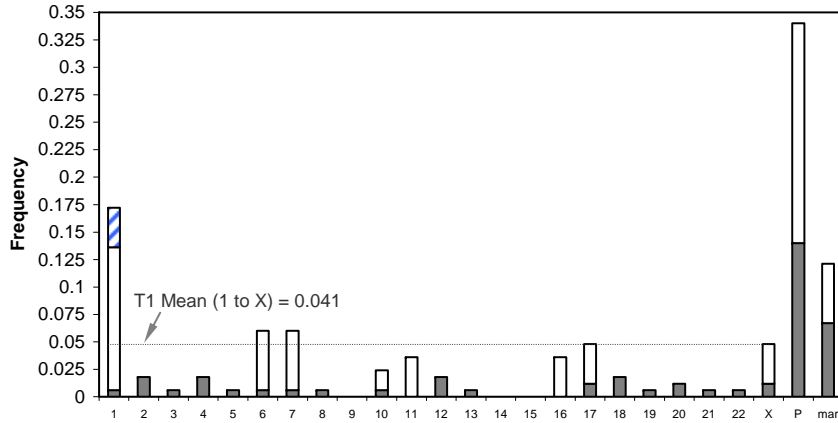


- **Karyotype analysis shows presence of altered karyotype in both untreated and treated Ad-SMC**
- **Altered karyotype frequency is within the range reported in healthy subjects**

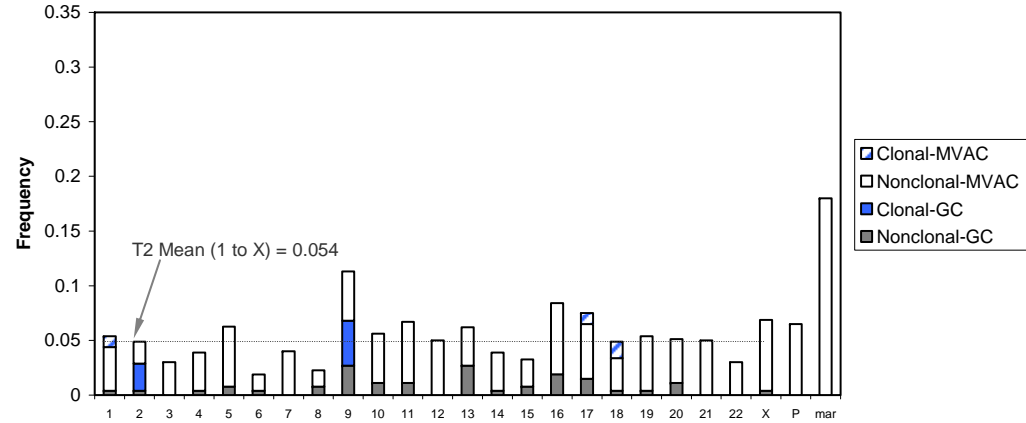
SMC Characterization

Karyotype – Frequency of Alterations

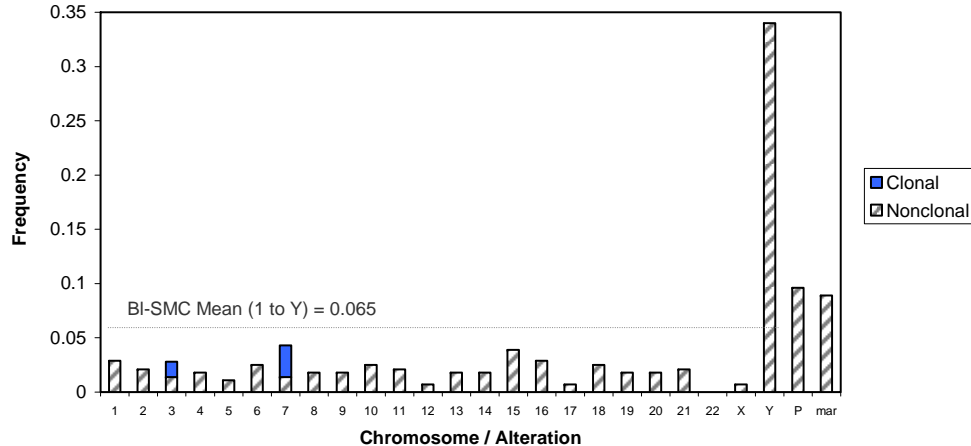
Ad-SMC - T1



Ad-SMC - T2



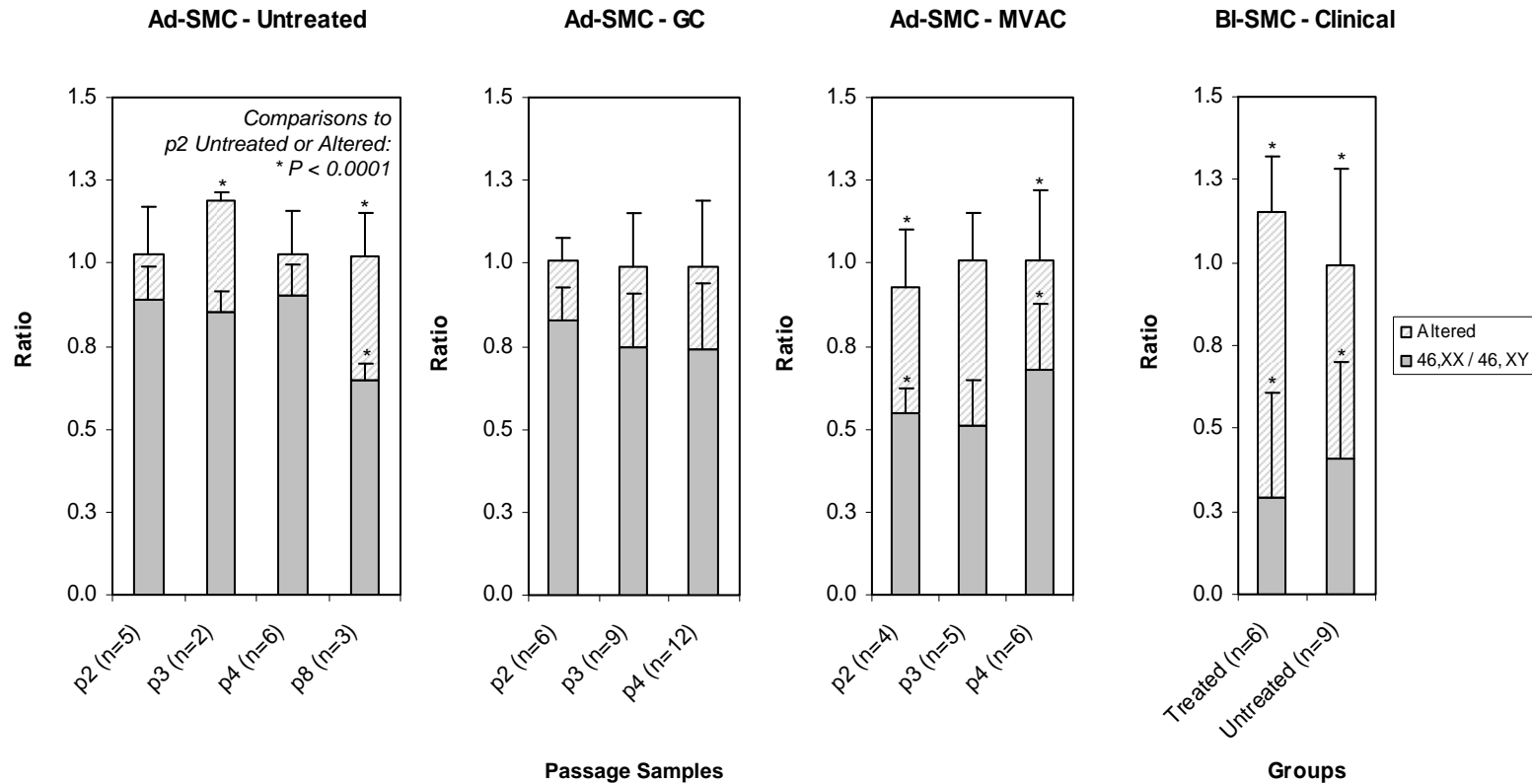
BI-SMC - Clinical



- **Altered karyotype frequency is donor tissue specific**
- **Altered karyotype frequency is similar for Ad-SMC and BI-SMC**

SMC Characterization

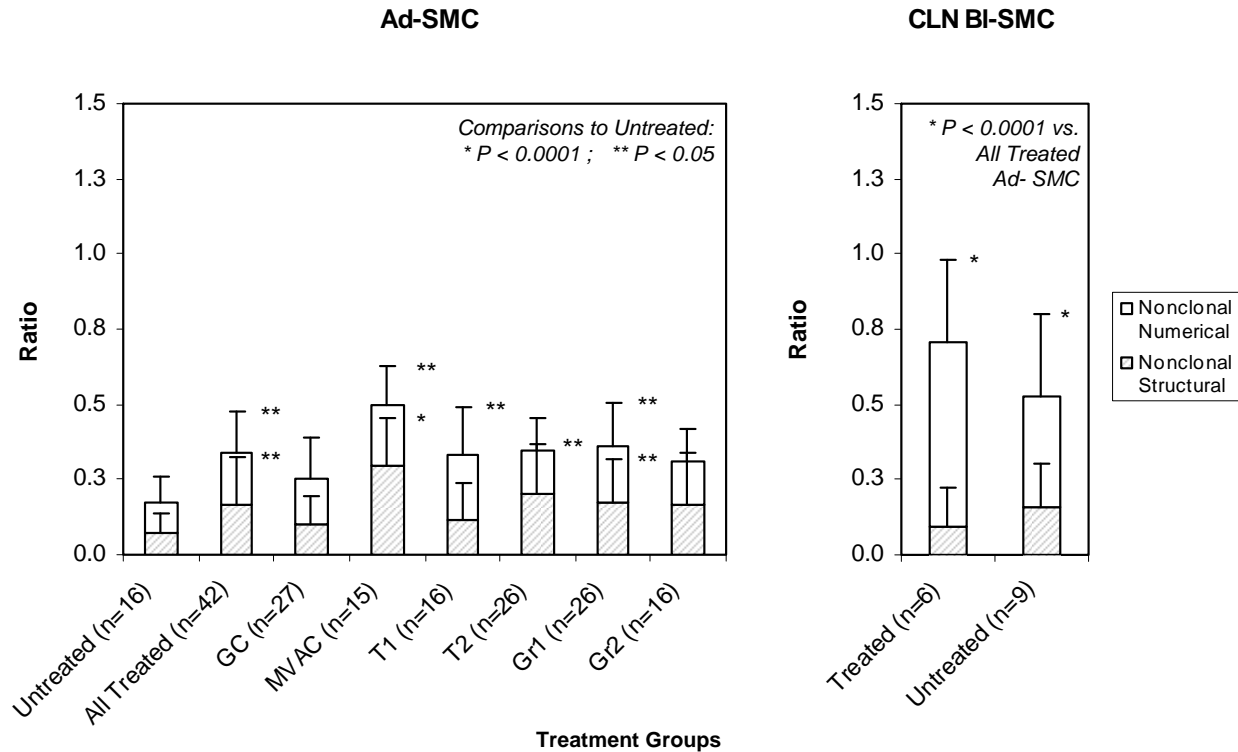
Karyotype – in Culture



➤ **Ad-SMC karyotype remains constant through cell expansion in culture**

SMC Characterization

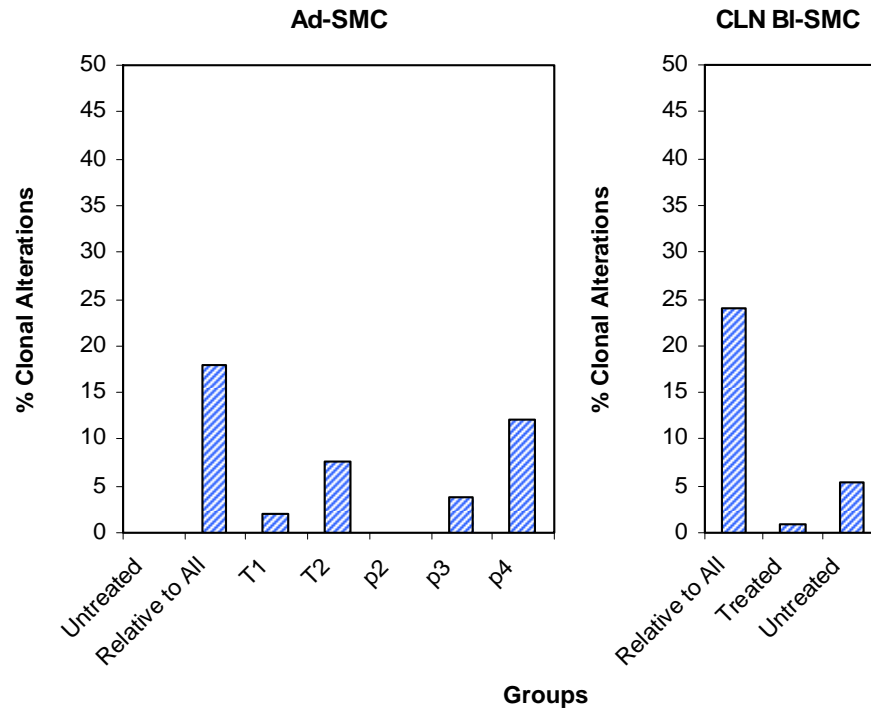
Karyotype – Nonclonal changes



- **Numerical & structural nonclonal changes are within reported ranges in literature**
- **Nonclonal changes are tissue and treatment dependent**

SMC Characterization

Karyotype – Clonal changes

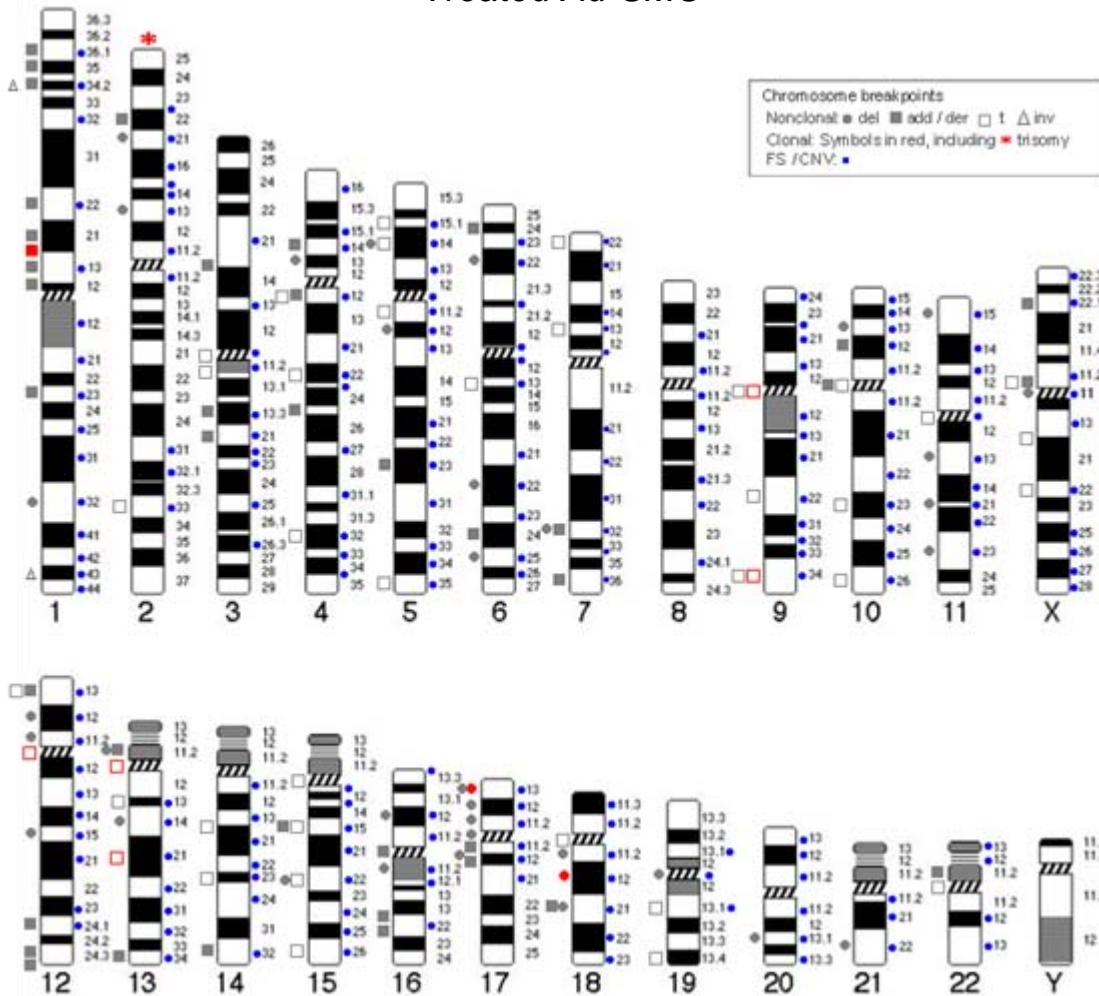


- *Clonal changes are within reported ranges in literature*
- *Clonal changes observed in in-vitro Treated Ad-SMC and in-vivo treated Clinical BI-SMC were similar*

SMC Characterization

Karyotype – Breakage Points

Treated Ad-SMC



- **Karyotype alterations occur at expected breakage points as reported in literature**
- **Treated in-vitro Ad-SMC show alterations at the same breakage points as in-vivo Clinical BI-SMC**

Conclusions

- ❖ ***In-vitro treated Adipose-derived SMC remain phenotypically and genetically comparable to in-vivo treated Bladder-derived SMC after exposure to chemotherapy drugs***
 - *Proliferative potential recovers after serial passages*
 - *Ploidy recovers within short-term culture*
- ❖ ***Although karyotype alterations are observed in treated samples, these are within the reported range of alteration for healthy individuals (frequency and breakage points)***
 - *Karyotype alterations remain constant (do not evolve) during cell expansion*
 - *Karyotype alterations are similar to those observed in samples from bladder cancer patients previously treated with chemotherapeutic drugs*
 - *In-vivo drug exposure in the clinical setting is expected to be 100 to 1000 fold less than those tested in-vitro in the current study*

These findings suggest that Ad-SMC exposed to chemotherapeutic agents in-vivo can be used in manufacture of SMC-based autologous regenerative products