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Genetic Stability of Autologous Human Smooth Muscle Cells

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Study Objective

Evaluate the genetic stability of SMC during ex vivo expansion

- *Use cytogenetic analysis to quantify ploidy and karyotype of adipose-derived SMC (Ad-SMC) in naïve healthy cell cultures used to engineer smooth muscle tissue for regenerative medicine applications*
- *Compare stability profile to SMC obtained from bladder cancer patients or exposed in vitro to chemotherapeutic drugs*

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SMC Stability
Sample Plan

	Ad-SMC	Bl-SMC
Untreated	*Ad* *Normal* Zen-Bio Inc. 28 to 51 years old 5F / 1M p0 to p4 n=39	*Unt-Bl* *Clinical* ClinicalTrials.gov 55 to 77 years old 1F / 3M p3 to p7 n=9
Treated	*Treated-Ad* *In vitro Model Drug (GC/MVAC)* Zen-Bio Inc. 25 & 44 years old 2F 1 to 3 p recovery n=66	*Treated-Bl* *Clinical (Chemoradiotherapy)* ClinicalTrials.gov 52 to 81 years old 5M p4 n=6

Genetic Stability:

- Ploidy
  (quiescent, proliferating cells)
- Karyotype
  (normal, altered chromosomes)

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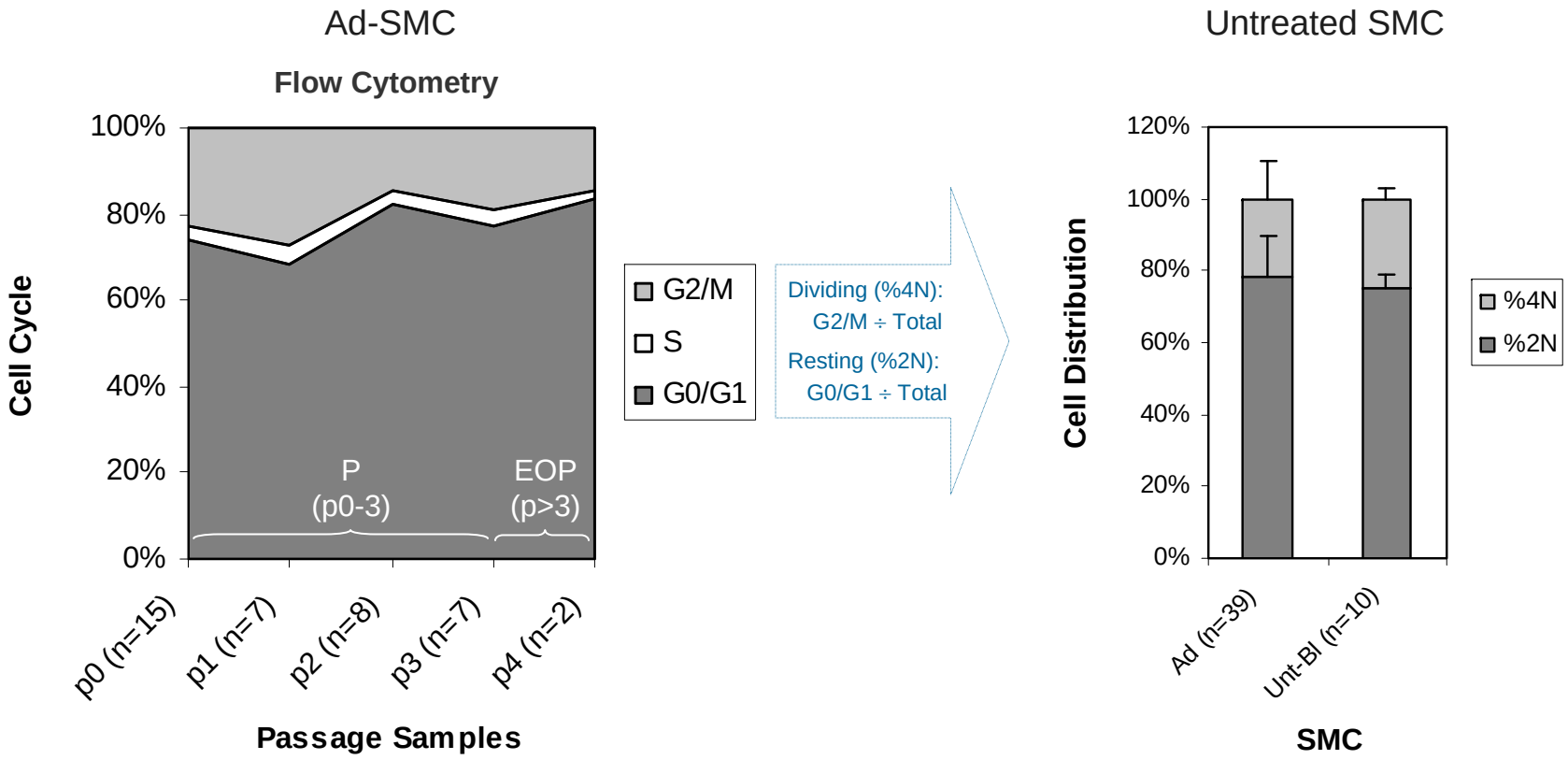
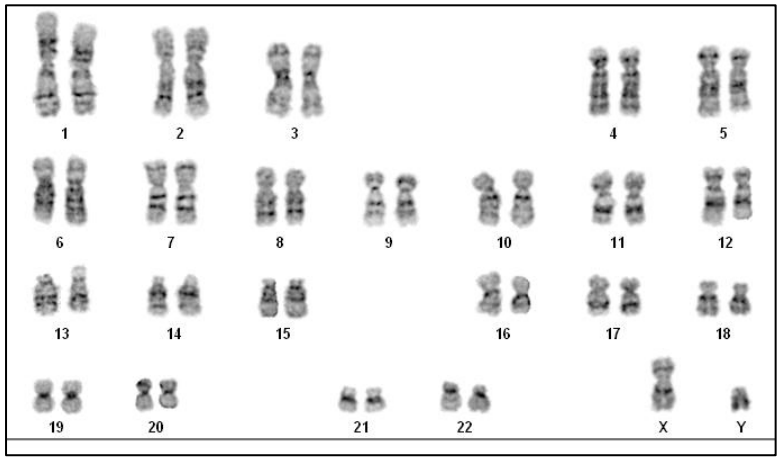
SMC Stability
Ploidy



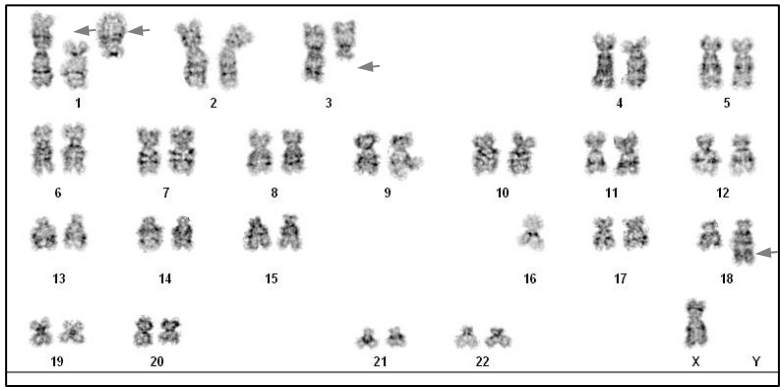
➢ ***Stable ploidy is maintained within production phase for SMC***

SMC Stability
Karyotype – Sample Analysis

G-banding (metaphases)



46,XY [1]



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

Sample Calculations

	Total	46,XY		Altered	Normal
Karyotype	No.	No.	Ratio	Ratio	Ratio
46,XY [20]	0	20 of 20	1.0	0	1.0
<4n> **[1]** /46,X,-Y **[1]** /46,XY **[18]**	2	18 of 20	0.9	0.10	1.0
46,X,-Y,del(1)(p21),+del(1)(p10), t(3;18)(q12;q23),-16 **[1]** /45,X,-Y, inv(7)(p15;q34) **[8]** /45,X,-Y **[10]** /46,XY **[1]**	31	1 of 20	0.05	0.95	0.05

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

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SMC Stability
Survey of Chromosomal Alterations

Normal
Age-related: • Random Gain or Loss • X,Y Loss (*e.g.*, frequency of 25%) • Polyploidy (*e.g.*, frequency of 0.5 to 20%) • Monosomy or Trisomy (*e.g.*, chromosomes 1,2,3,4,6,7,15,18) Other: • Mixed changes (*e.g.*, frequency of 0.7 to 20%; 84% numerical, 16% structural, 5% polyploidy) • Fragile Sites (*e.g.*, most active at cytogenetic loci 1p31, 3p14, 3q13.3, 16q23.2) • Copy Number Variants (*e.g.,* common amplifications or deletions at 1q, 7p, 9p, 13q, 17p, 20q)

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

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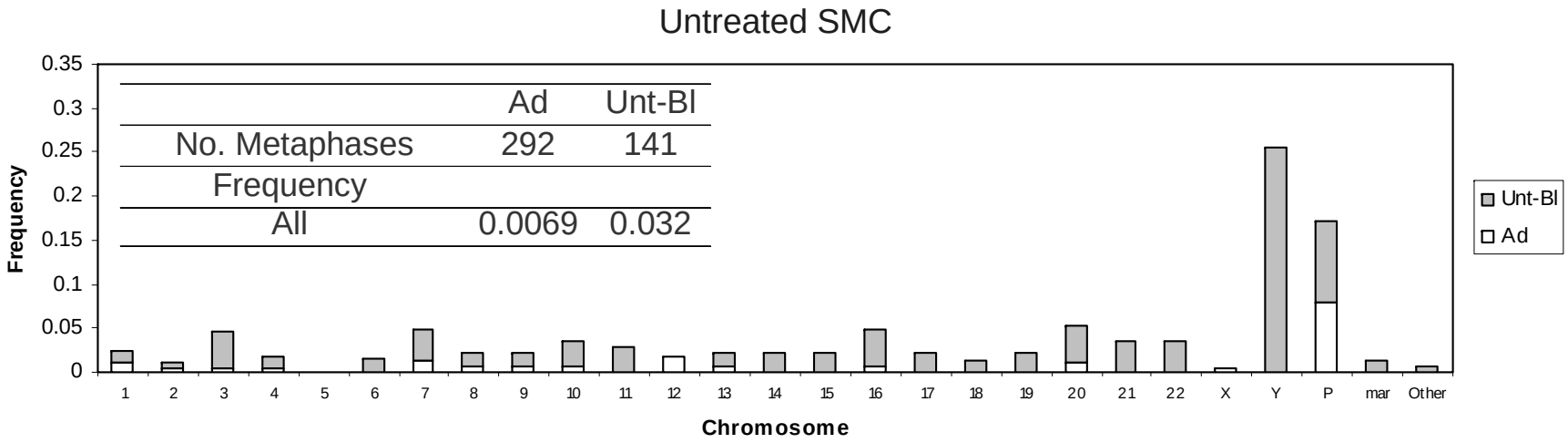
SMC Stability
Karyotype – Frequency



- ***Analysis shows presence of expected chromosomal changes in both untreated Ad-SMC and Clinical Bl-SMC***
- ***Altered karyotype frequency is within the range reported in healthy subjects***

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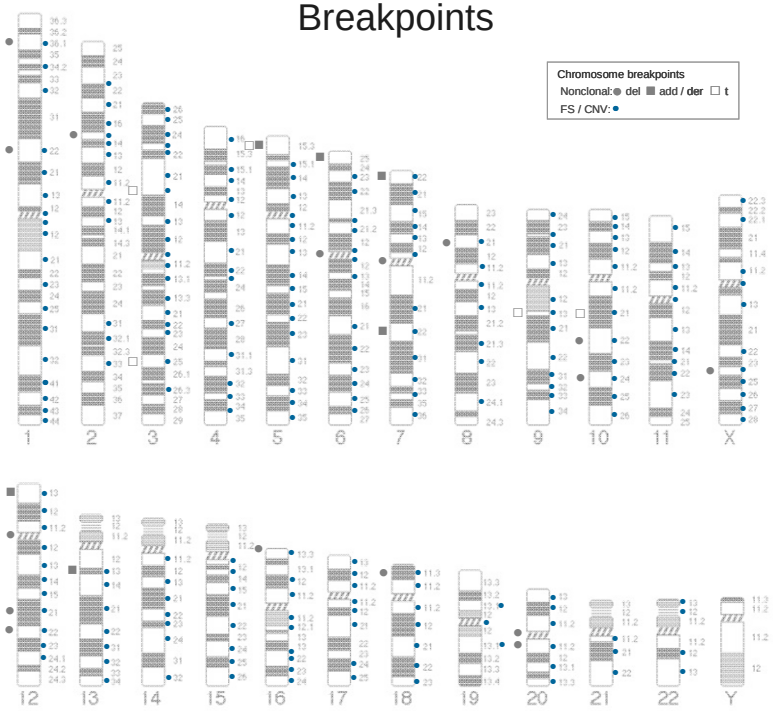
SMC Stability
Karyotype – Details (Ad-SMC)

Breakpoints



Structural & Numerical

No.	List	Total
1	del(p22)[2], +del(p36)[1]	3
2	del(p15)[1]	1
3	t(3;4)(q25;p16)[1]	1
5	add (p15.3)[1], der(p16)[1], t(5;10)(p15.3;q21.2)[1]	3
6	add(p25)[1], add(qter)[1], del(q11.2)[1]	3
7	del(p11.1)[1], add(p22)[1], add(q22)[1], +[1]	3
8	del(p21)[1]	1
9	t(9;11)(q13;pter)[1], ?del(q22)[1]	2
10	?del(q22)[1], del(q24)[1]	2
11	+[1]	1
12	del(p11.1)[1], add(pter)[1], del(q21)[1], del(q22)[1]	4
13	der(q13)[2]	2
16	del(p13)[1], +[1]	1
18	?del(p11.3)[1], +[1]	1
20	del(p11.1)[1], del(q11.2)[1], -[1]	2
X	del(q24)[1]	1
Total		31

- ***Alterations occur at expected breakage points, and numerical changes are random, as reported in literature***

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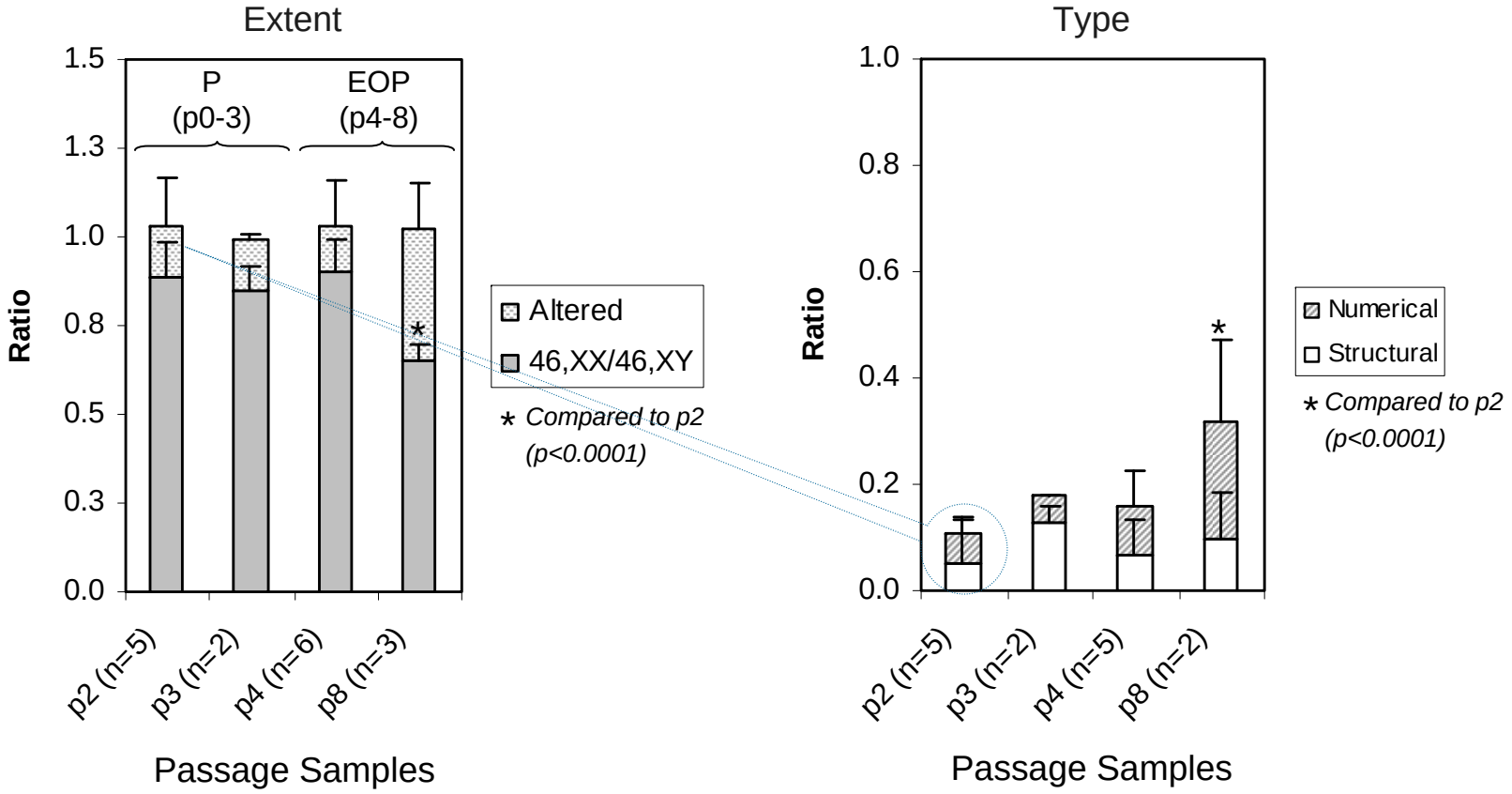
SMC Stability

Karyotype – Changes (Ad-SMC)



- ***Karyotypic stability is maintained through production phase***
- ***Numerical & Structural changes are within reported ranges in literature***

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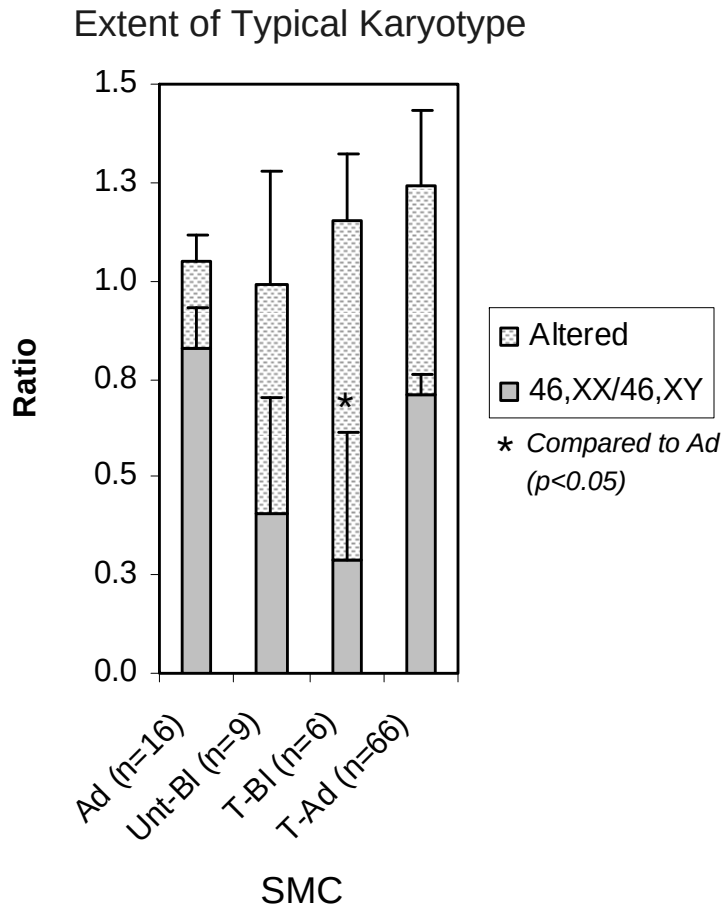
SMC Stability
Karyotype – Comparison



- ***Extent of typical karyotype is greater in Ad-SMC***
- ***Impact of change is larger in Bl-SMC***
  - ✓ *Unrelated to expected chromosomal alterations in urothelial cells in bladder cancer*

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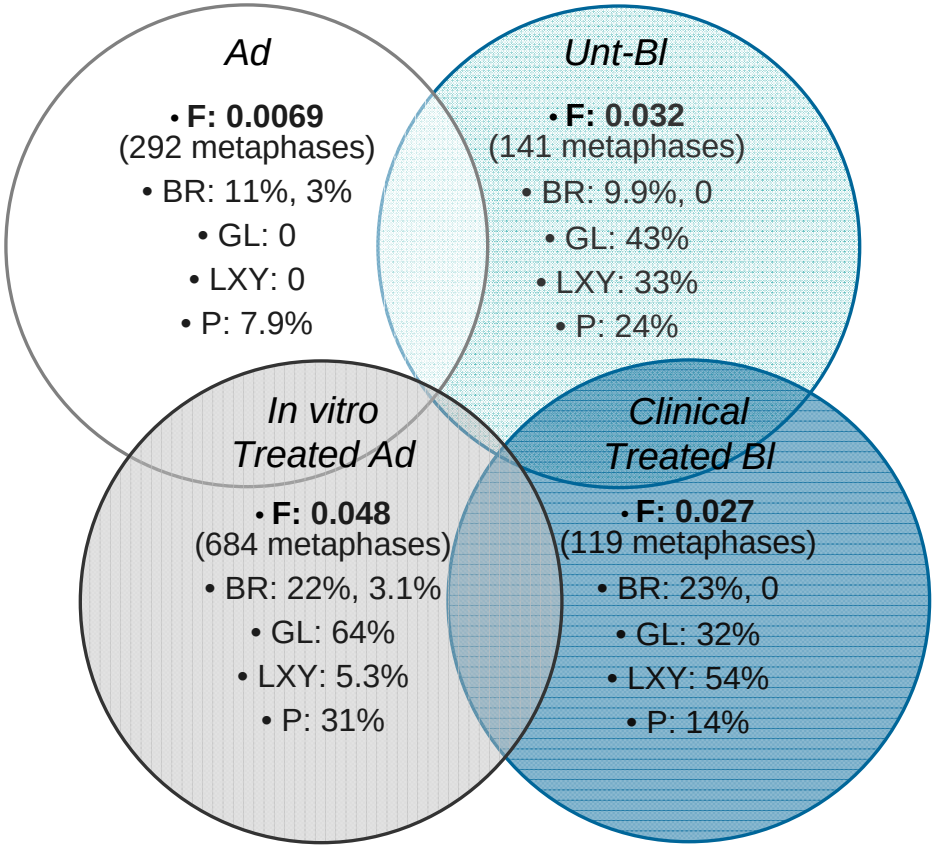
SMC Stability
Karyotype – Summary



F: Frequency

BR: Breaks, Recurrence

GL: Gain/Loss

LXY: Loss (X,Y)

P: Polyploidy

➢ ***Extent of chromosomal changes evolve along with age and exposure to cancer drugs***

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Conclusions

- *Ex vivo expansion of adipose- and bladder-derived SMC maintains their genetic stability*
  - *Ploidy confirms their proliferative potential*
  - *Karyotype confirms their cytogenetic health*
- *Although karyotype alterations are observed, these are normal and within the reported range of alteration for healthy individuals (frequency and breakage points)*
  - *Karyotype alterations reflect a history of replicative stress (*e.g.*, due to age, disease, cancer treatment)*

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

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