TELOMERE DYNAMICS IN ANEUPLOIDY AND CANCER: A TRANSLATIONAL RESEARCH

Sen Pathak, Ph.D., F.N.A.Sc.
Distinguished Research Professor
Department of Genetics
The University of Texas M. D. Anderson Cancer Center
Houston, Texas 77030, U.S.A.
E-mail: spathak@mdanderson.org

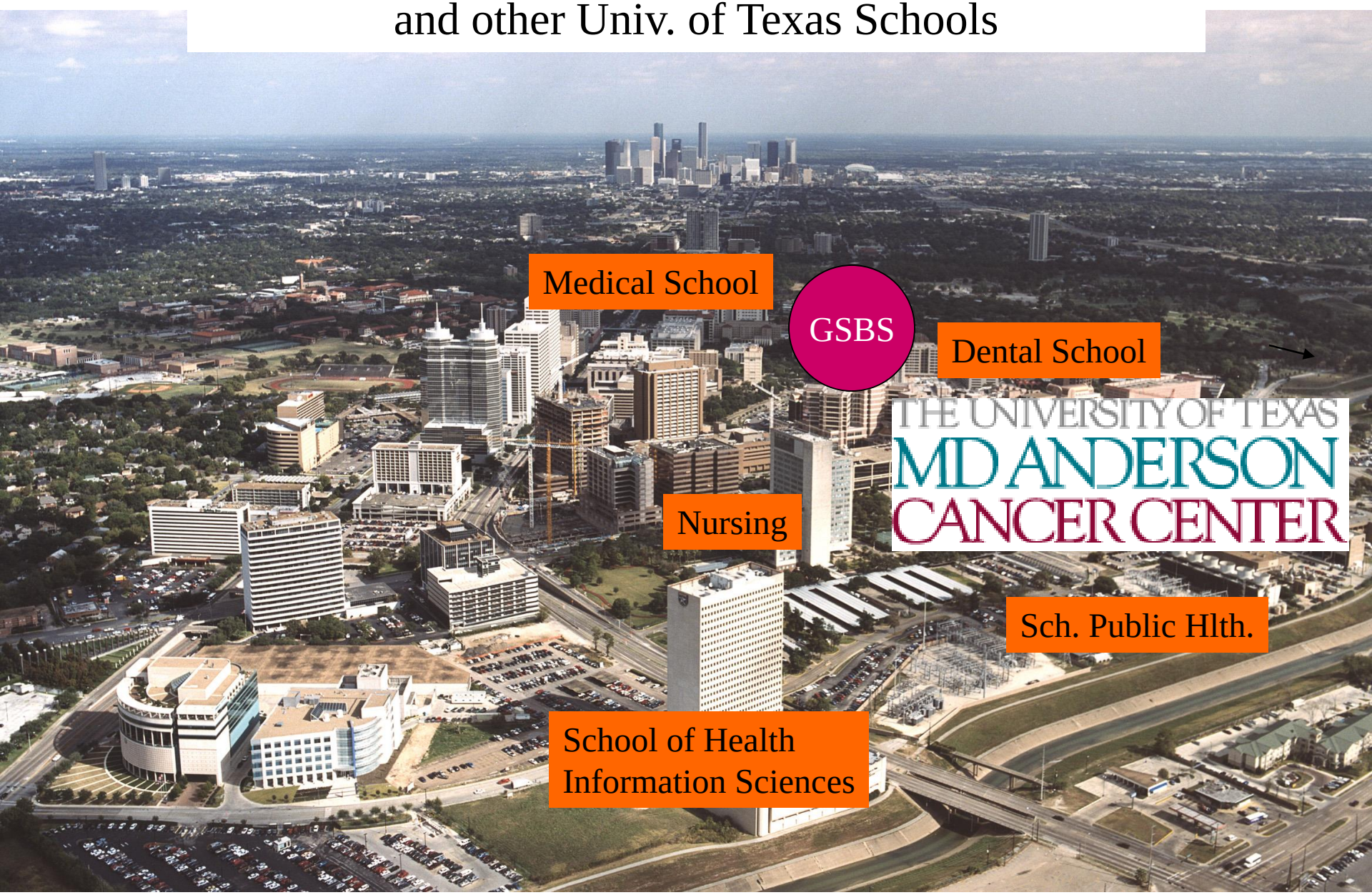

Graduate School of
Biomedical Sciences –
M. D. Anderson Cancer Center
The “Global” View

Satellite Photo “Zooming” in on the Texas Medical Center and M D Anderson

Texas Medical Center

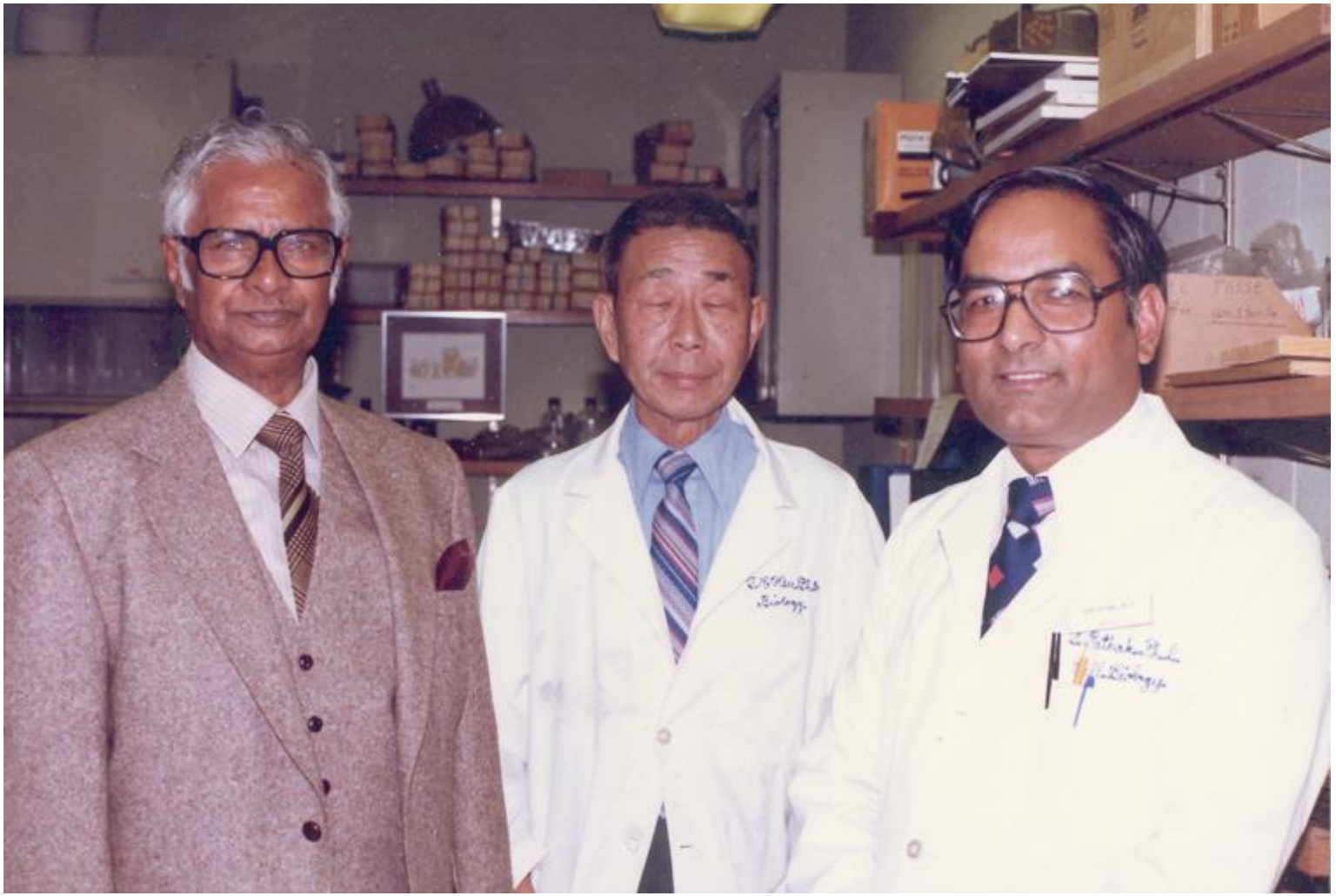

THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER

GSBS is a Collaborative Institution between MDACC and other Univ. of Texas Schools



M.D. Anderson Hospital & Tumor Institute, 1970

M. D. Anderson Cancer Center, 2002

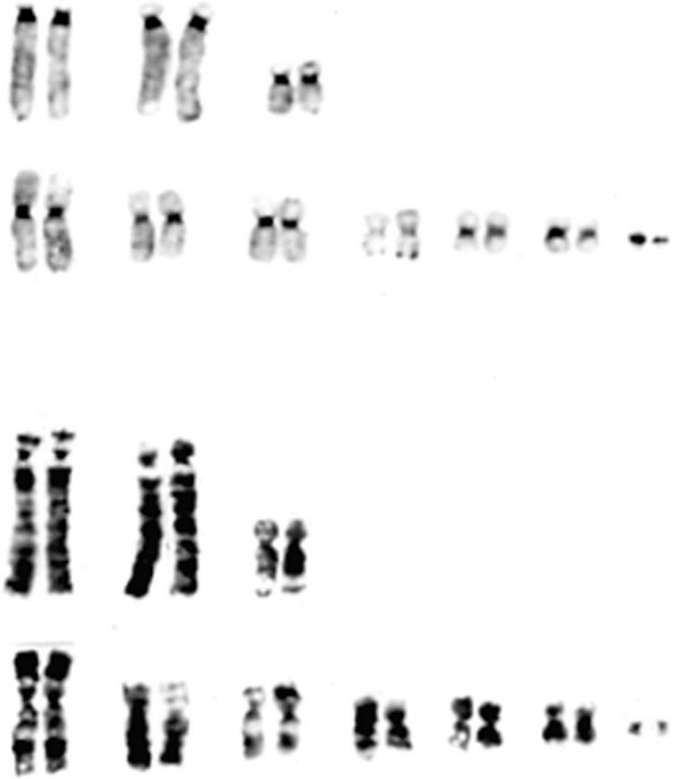


S. P. Ray-Chaudhuri, T. C. Hsu and S. Pathak, 1980

Hybrid Tylomys Karyotype

Pathak S. et al., Chromosoma, 1973.

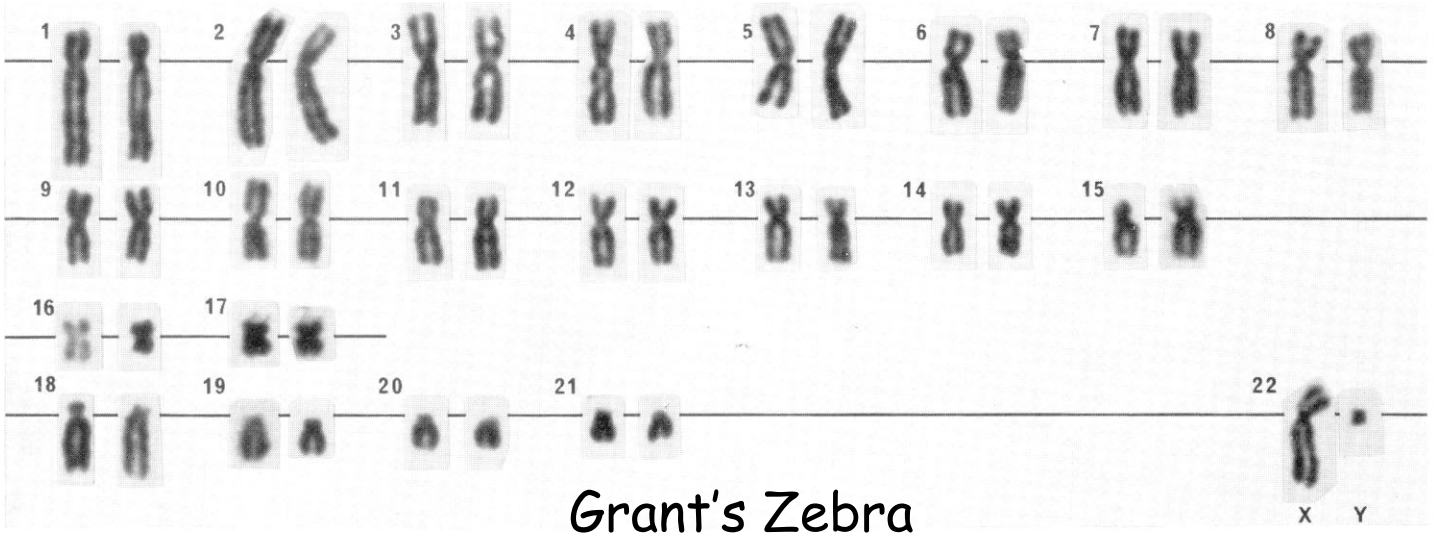
Aardvark



Pathak et al., 1980

2n = 20

2n = 44



Grant's Zebra

“The chromosomes never lie”

Pathak, S., 1972

Origin of Species and Cancer

- Survive
- Protection from natural predators
- Plenty of food and water supplies
- Migration
- Prolific breeder

- Survive
- Protection from natural predators
- Plenty of growth and angiogenic factors
- Metastasis
- Prolific cell division

Cancer Development

INTERACTION (S)

GENETIC BACKGROUND (Genotype)

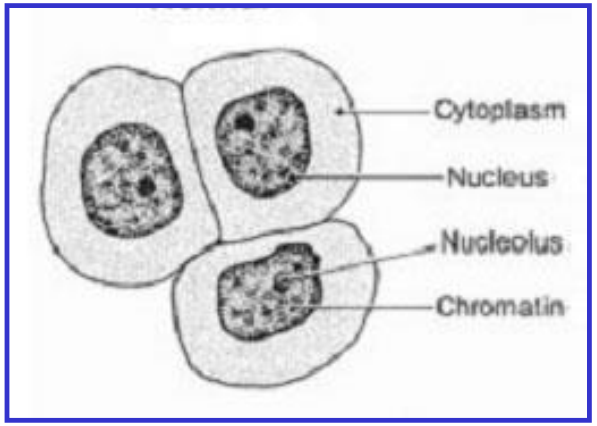
ENVIRONMENTAL EXPOSURE (Internal & External)

CANCER

What is cancer?

Normal cells vs. cancer cells

Normal cells:



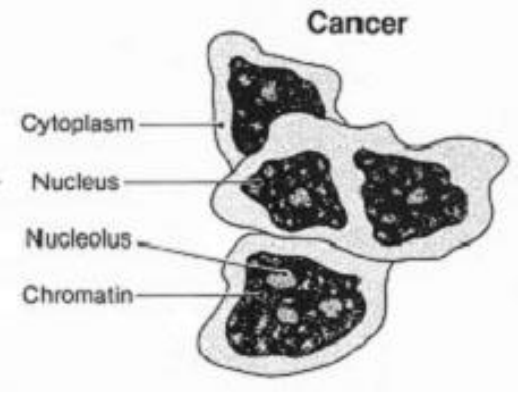
- exhibit orderly, controlled growth
- repair damaged DNA
- produce new cells only when needed
- undergo a genetically programmed cell death

What is cancer?

Normal cells vs. cancer cells

Cancer cells:

- grow in uncontrolled, patterns
- display unregulated cell division



- are unable to perform their specialized functions
- grow beyond the boundaries of normal tissue
- fail to die at genetically regulated time

Cancer originates in the organ/tissue-specific stem cells

- **The development of individual organs in human embryos involves the formation of tissue-specific stem cells.**
- **Only stem cells participate in organ/tissue homeostasis by replacing old somatic cells lost as a result of aging, injury or disease.**
- **Only cycling (stem) cells accumulate mutations; non-dividing do not. > 95 % human cancers are sporadic.**
- **Established cell lines from human and mouse tumors have their own cancer stem cells.**

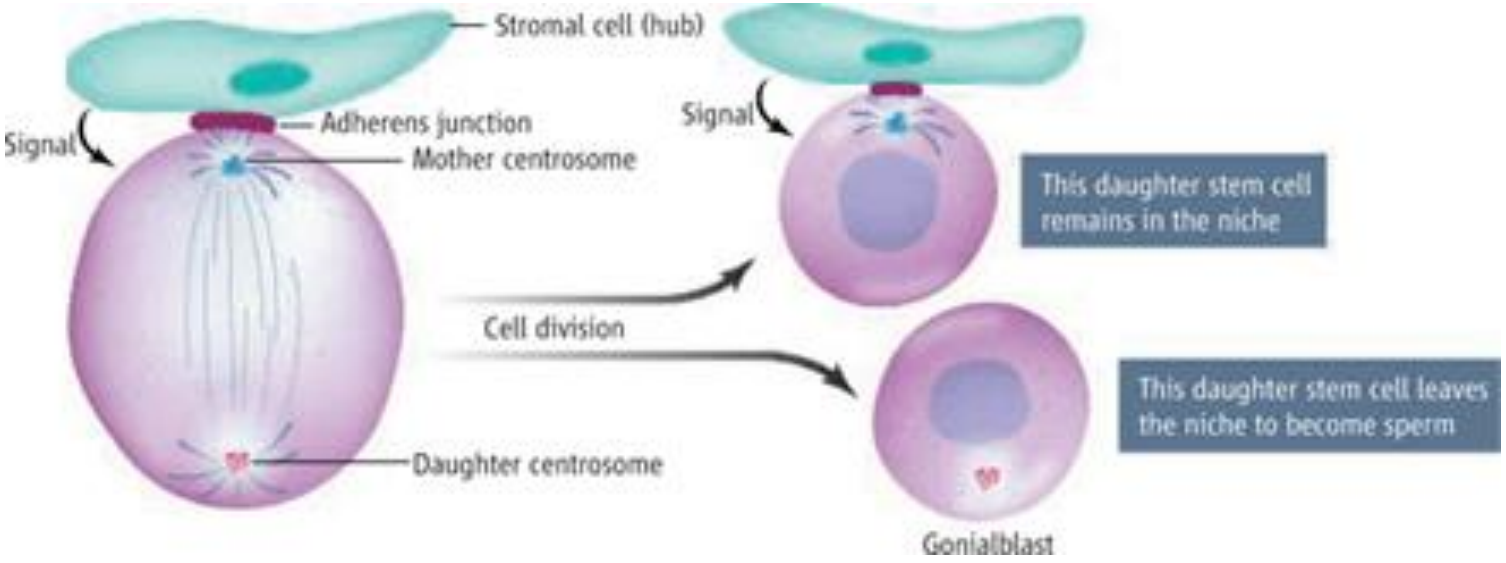
Similarities between Normal Stem Cells and Cancer Stem Cells

Normal stem cells

- **Self renewal**
- **Migrate**
- **Differentiate**
- **Proliferate indefinitely**
- **Are heterogeneous with different phenotypes**
- **Express telomerase**
- **Are tissue –specific**
- **Have extended telomere**
- **Undergo organogenesis**
- **Undergo apoptosis**

Cancer stem cells

- **Self renewal**
- **Metastasize/migrate**
- **Differentiate**
- **Proliferate indefinitely**
- **Are heterogeneous with different phenotypes**
- **Express telomerase/ALT**
- **Are tissue –specific**
- **Have extended telomere**
- **Undergo limited organogenesis**
- **Undergo apoptosis**

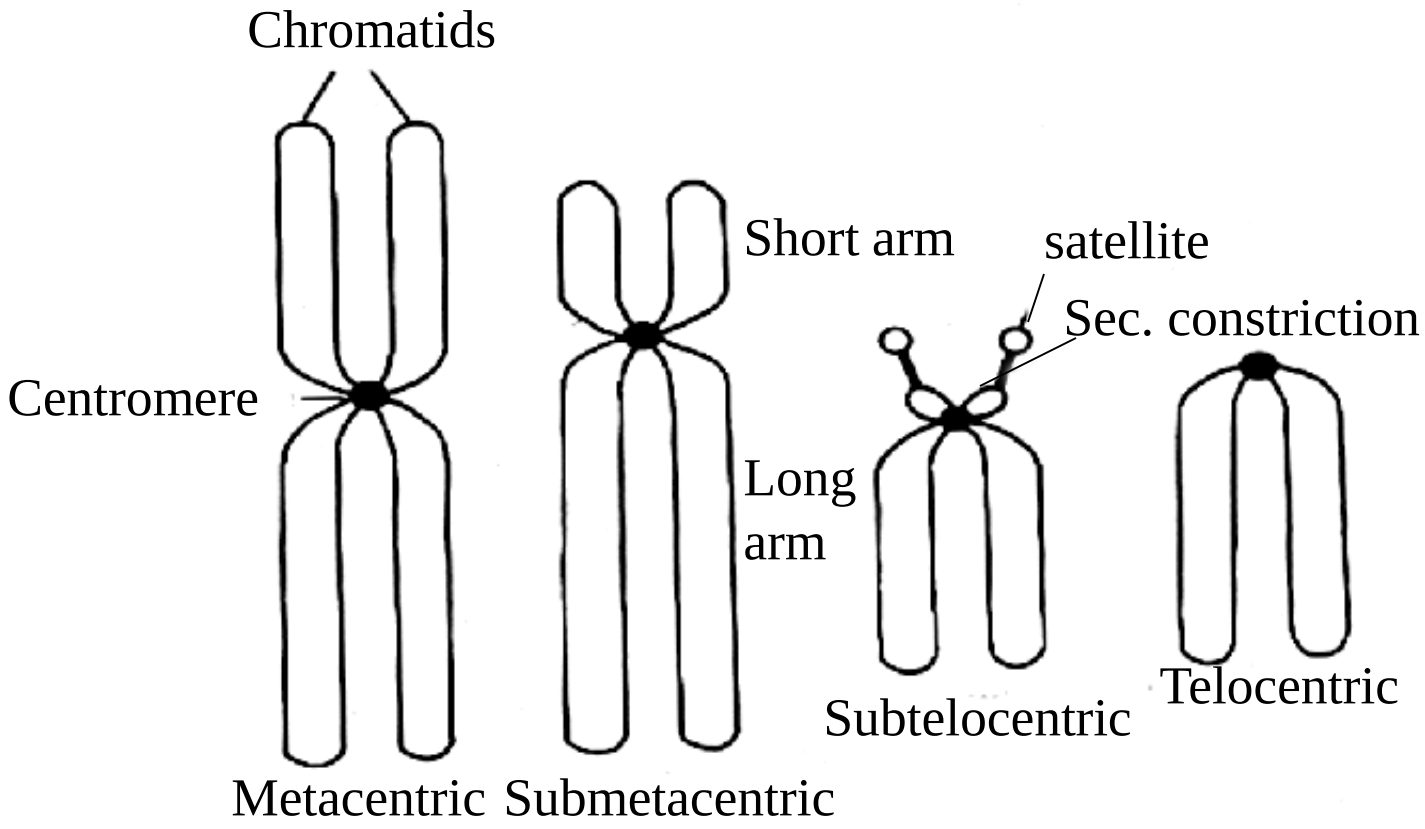


The Mother of All Stem Cells?

Spradling AC & Zheng Y., Jan. 26,Science Vol: 315, 2007

The Dynamic Chromosome

- Chromosomes evolve by modification, translocation, deletion and recombination of genetic material.
- Relating these dynamic (evolutionary) changes to the functional biology of the chromosome and now the telomere biology remains an even greater challenge.


Chromatids
Centromere
Short arm
satellite
Sec. constriction
Long arm
Metacentric
Submetacentric
Subtelocentric
Telocentric

H. J. Muller

Barbara McClintock

Functions of telomeres

- Telomeres protect the chromosome ends from degradation and fusion.
- Telomeres mask chromosome ends from DNA - damage response that might trigger senescence and apoptosis.
- Telomeres help position the chromosomes in the nucleus.
- Telomeres provide means of maintaining chromosome length through many generations of replication.
- Telomeres initiate chromosome pairing in meiosis.

Telomere: A specialized DNA-protein complex at the tips of linear chromosomes.

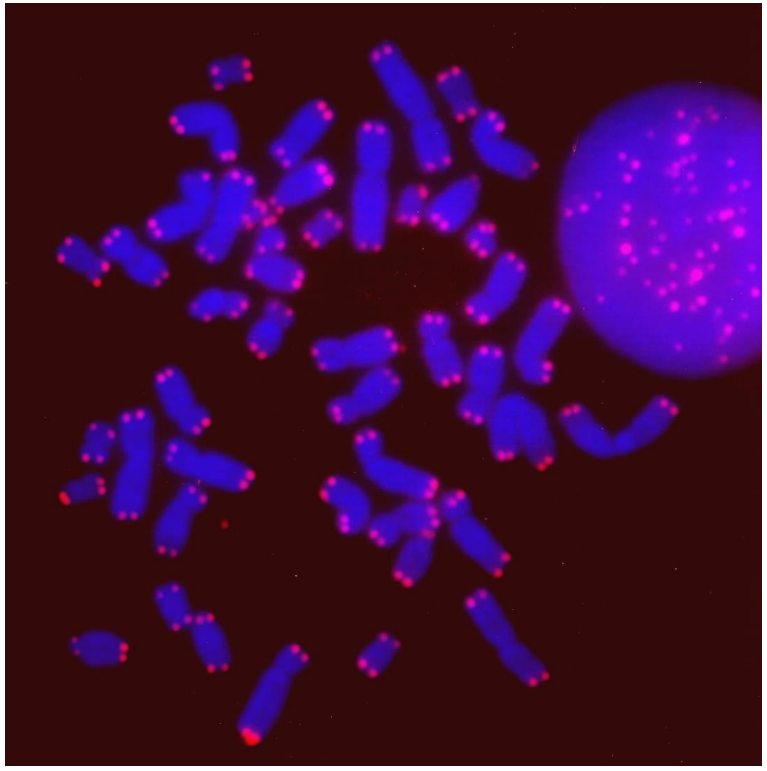
Mammalian telomere DNA consists of tandem repeats of TTAGGG plus a variety of attached proteins. The telomeres have a single-stranded 3' overhang

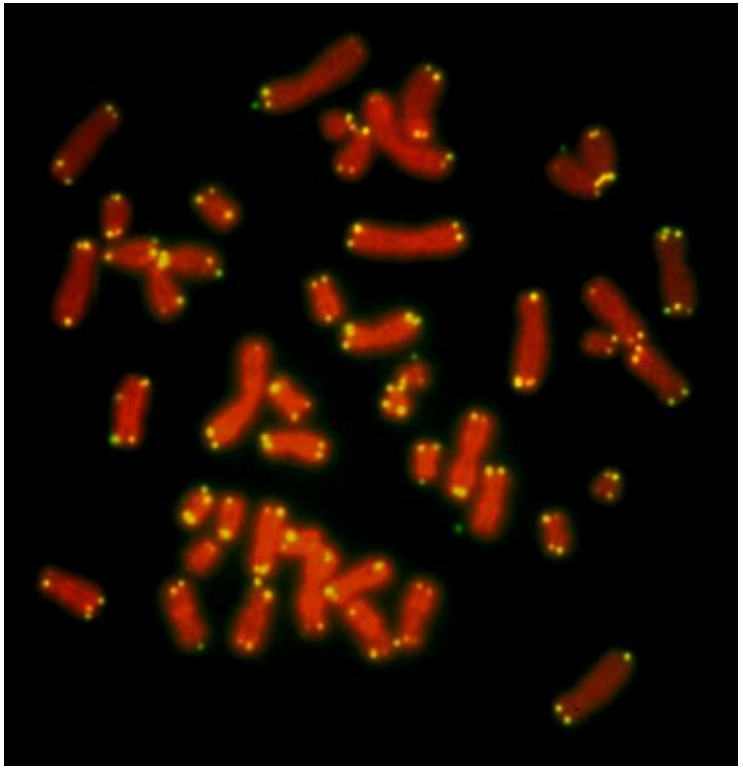
5'-TTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG-3'
3'-AATCCCAATCCCAATCCCAATAAAAATCCC-5"

The average length of the double-stranded TTAGGG repeat varies among species:

Mice ---------- 50,000 bp
Man ---------- 10,000 bp
Cattle ---------- 13,000 bp to 20,000 bp

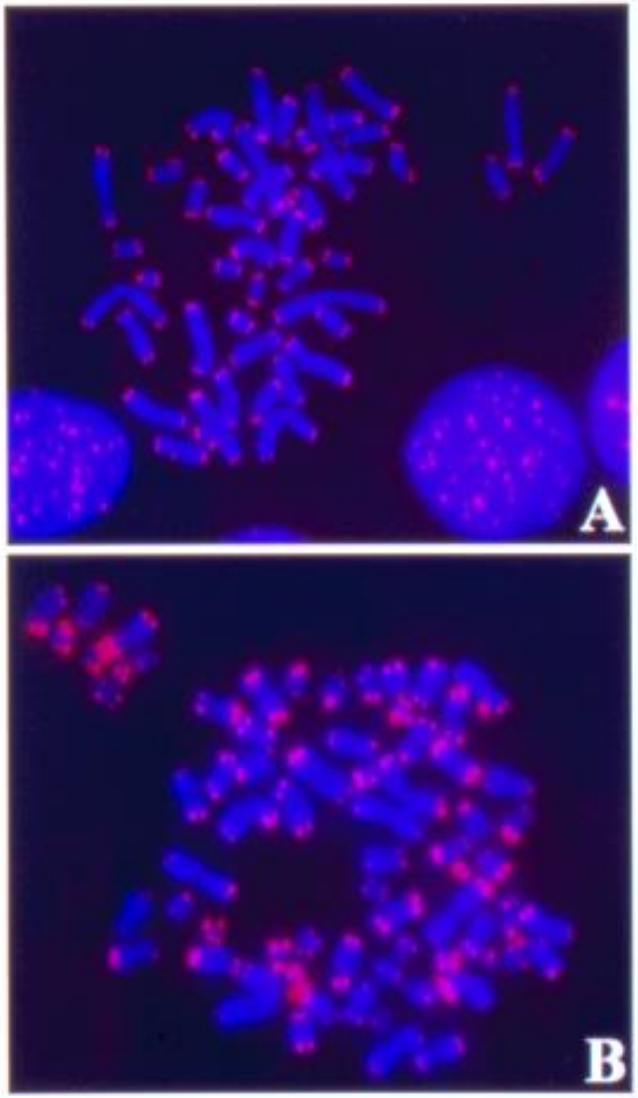
The single-stranded portion is believed to loop back and attach to the double-stranded portion to from what is referred to as a t-loop.





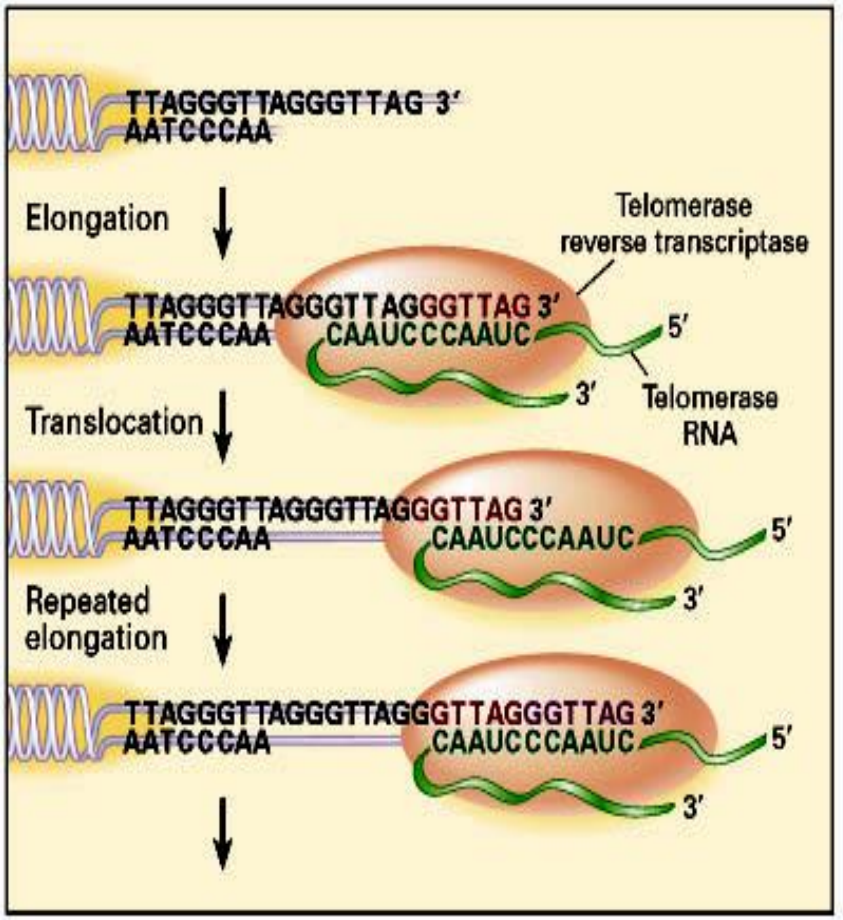
Mechanisms of Telomere Length Maintenance

- **Telomerase-mediated; common in cancer cells, slow process, less heterogeneity in telomeres**
- **Recombination-mediated or Alternative lengthening of telomere (ALT); rare in cancer, sudden increase, great heterogeneity of telomere size (ranging from undetectable to abnormally long) within individual cells, presence of nuclear bodies containing extrachromosomal telomeric DNA**
- **Both mechanisms operating simultaneously in some tumors**

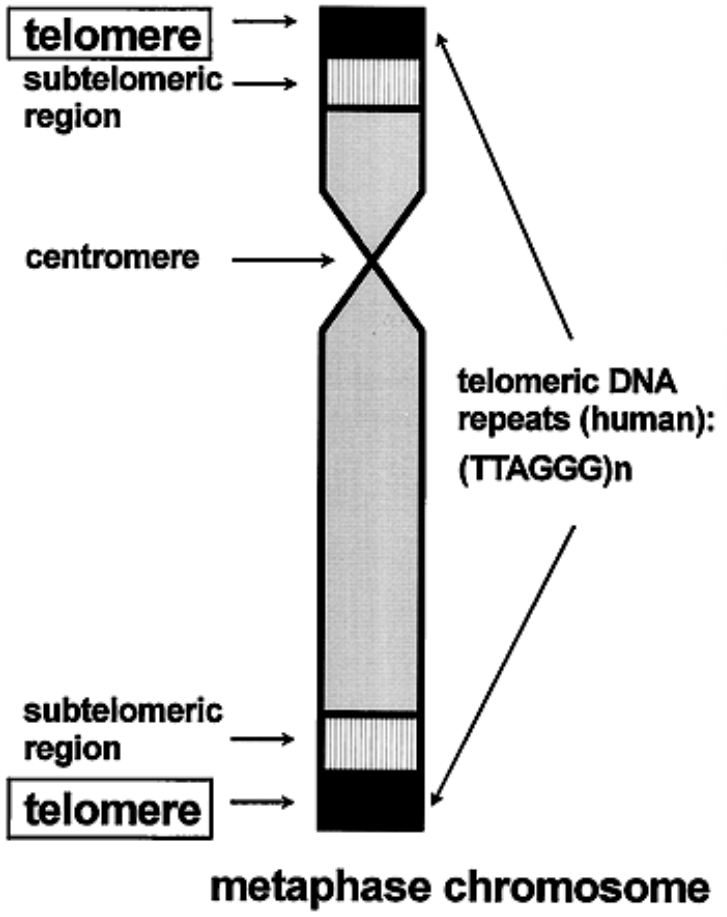

A
B

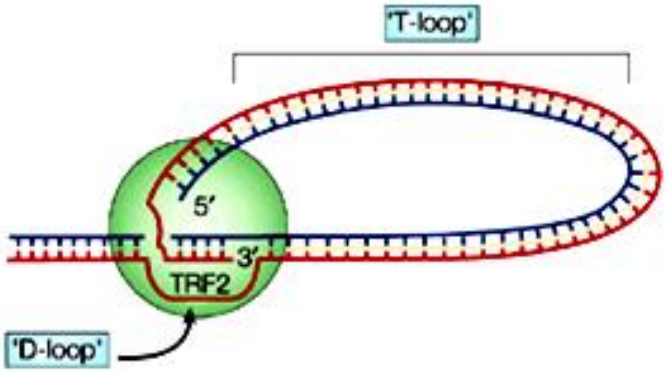
Telomerase= TERT+TERC

- Ribonucleoprotein complex
  - Telomerase reverse transcriptase (TERT)
  - RNA template (TERC)
- Expression is negatively regulated: human somatic cells do not express telomerase, resulting in telomere shortening.
- *Adds de novo* telomeric repeats

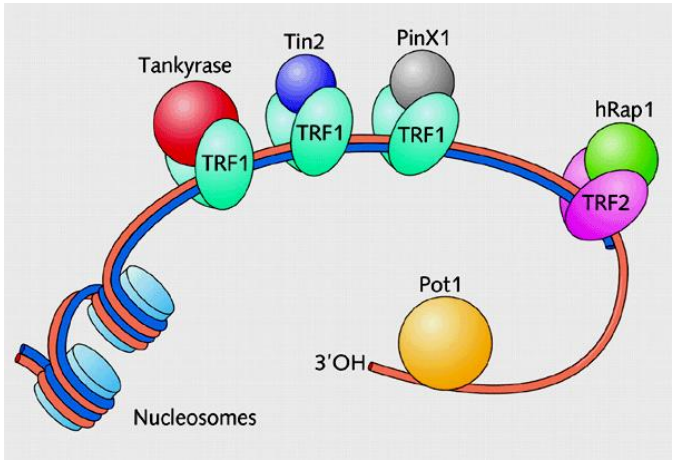


Telomere conformations





Closed conformation

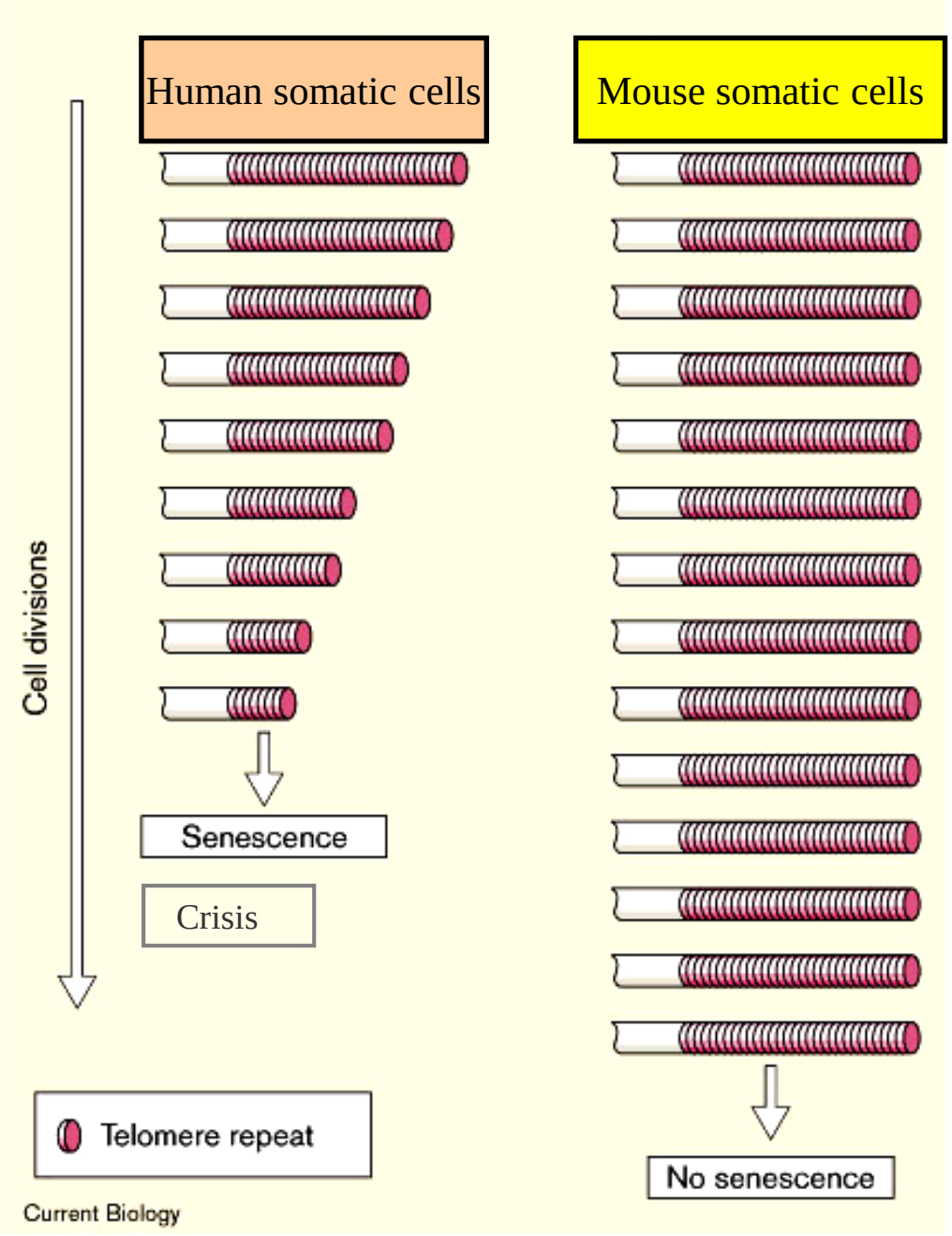


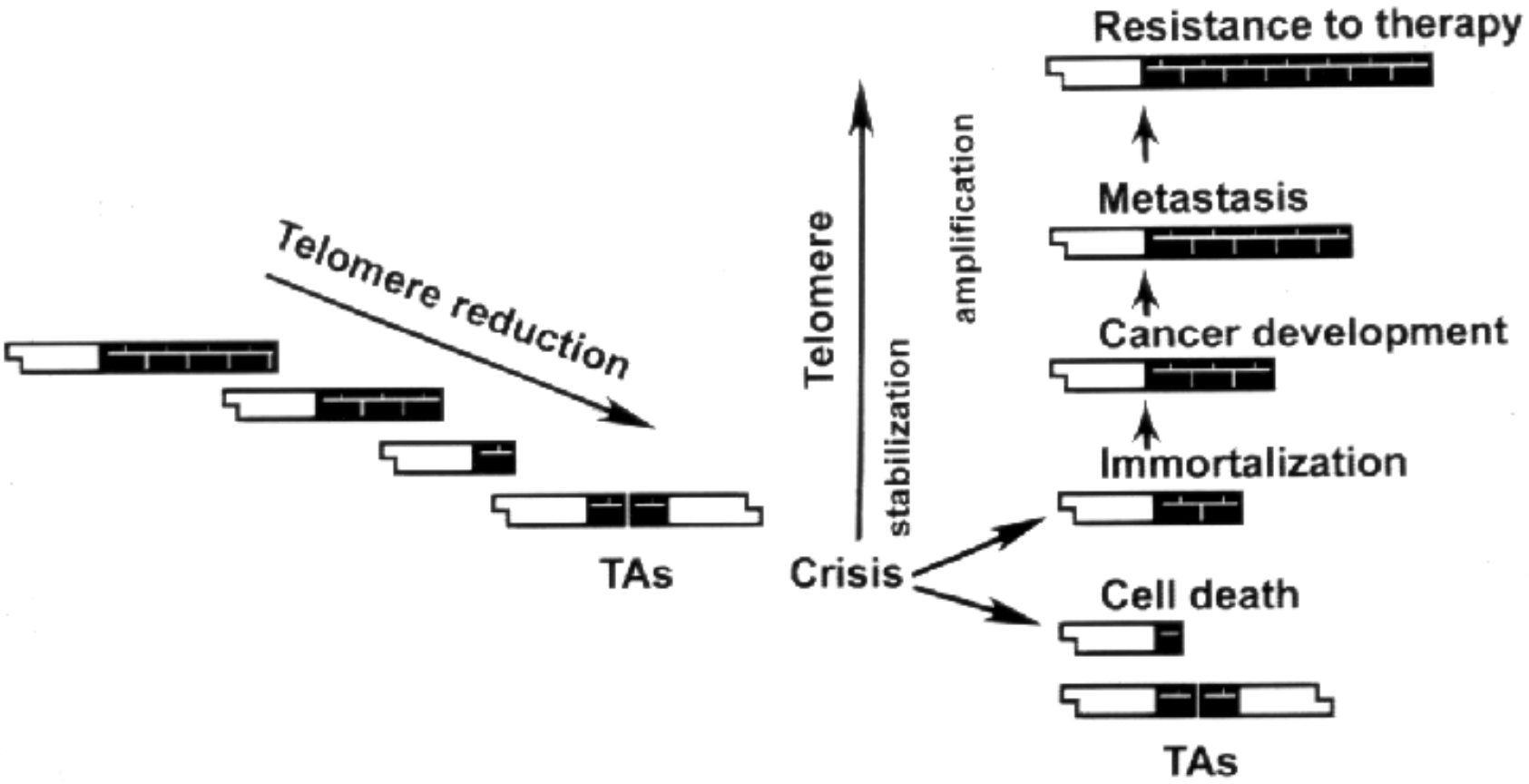
Open conformation

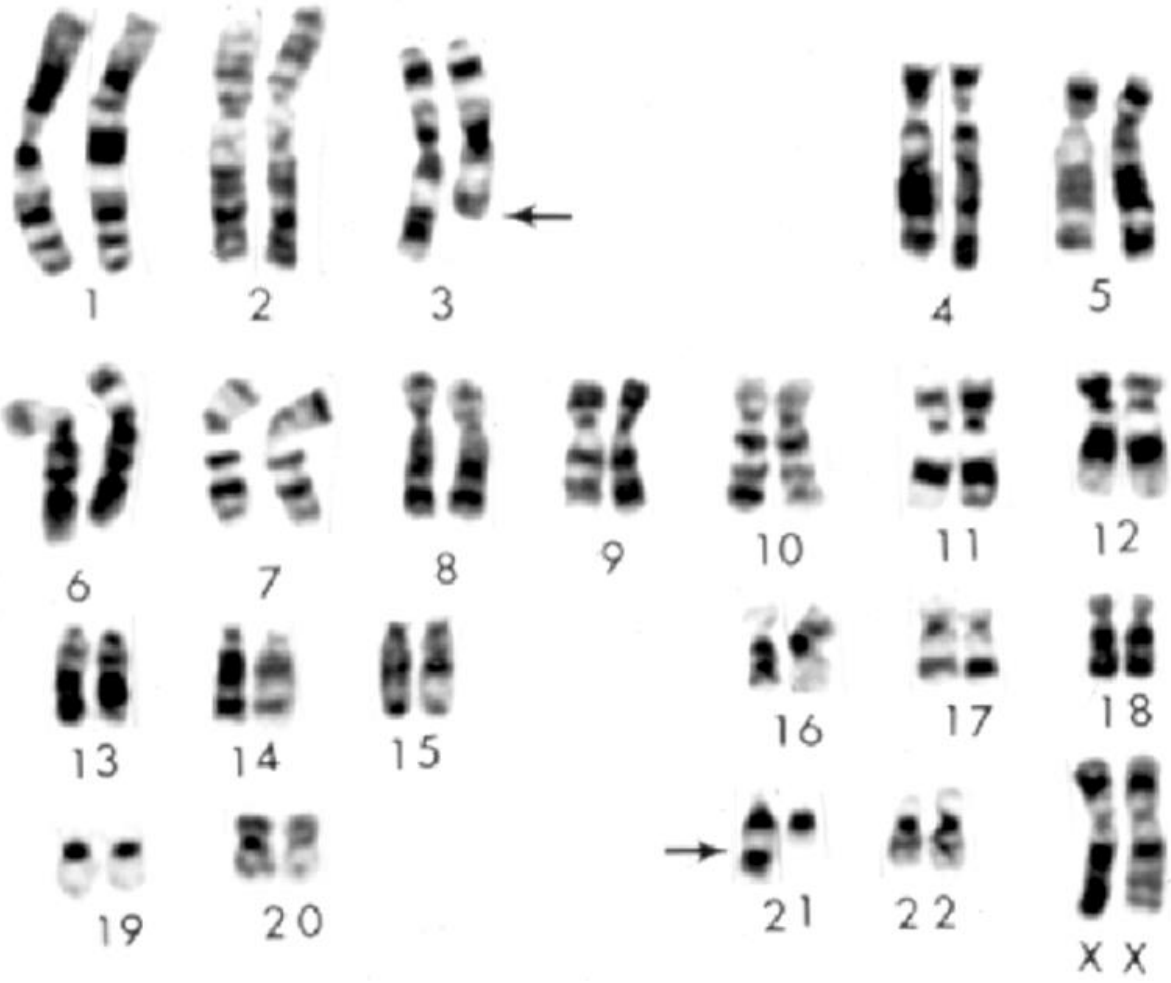
DNA replication?

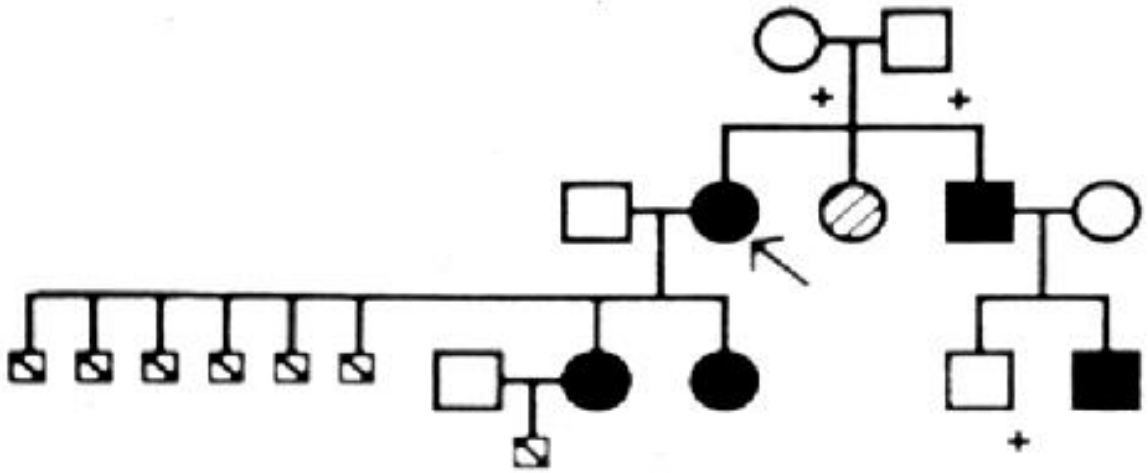
A specialized DNA protein Complex at the tip of linear chromosomes

Differences in telomere length regulation between human and mouse




Resistance to therapy
Metastasis
Cancer development
Immortalization
Cell death
TAs
Telomere reduction
TAs
Crisis
Telomere
stabilization
amplification


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X X



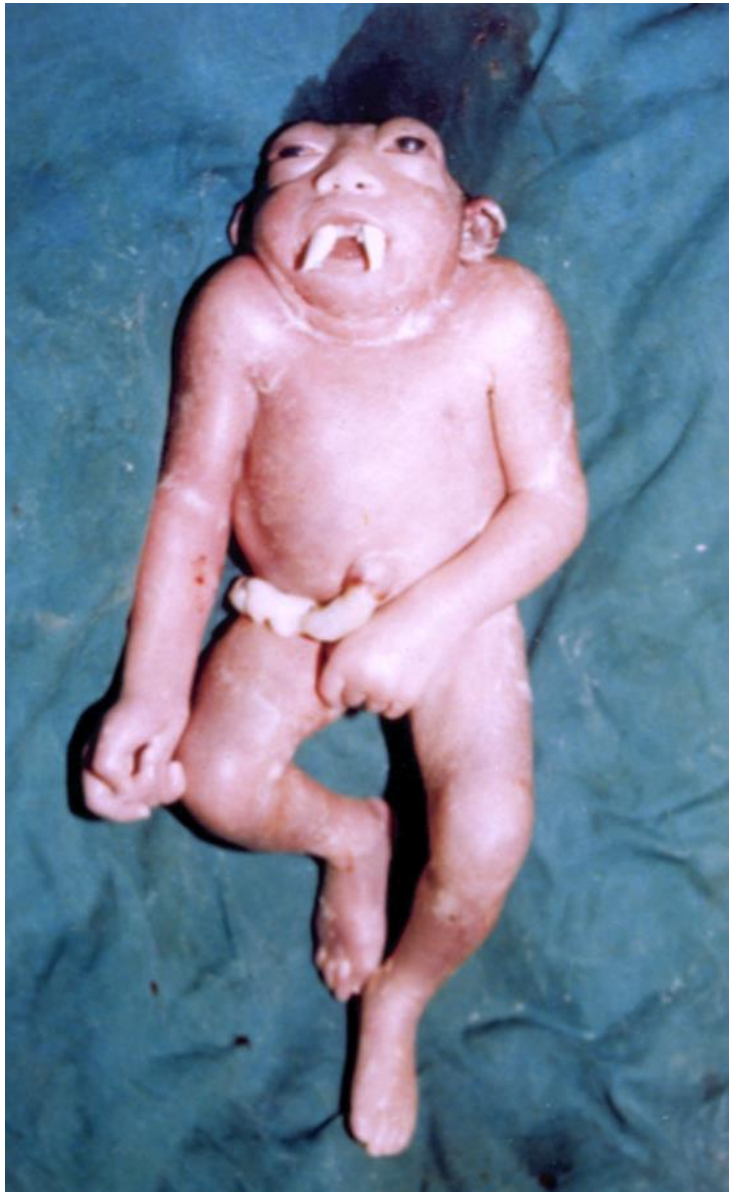
Bearer of chromosome anomaly

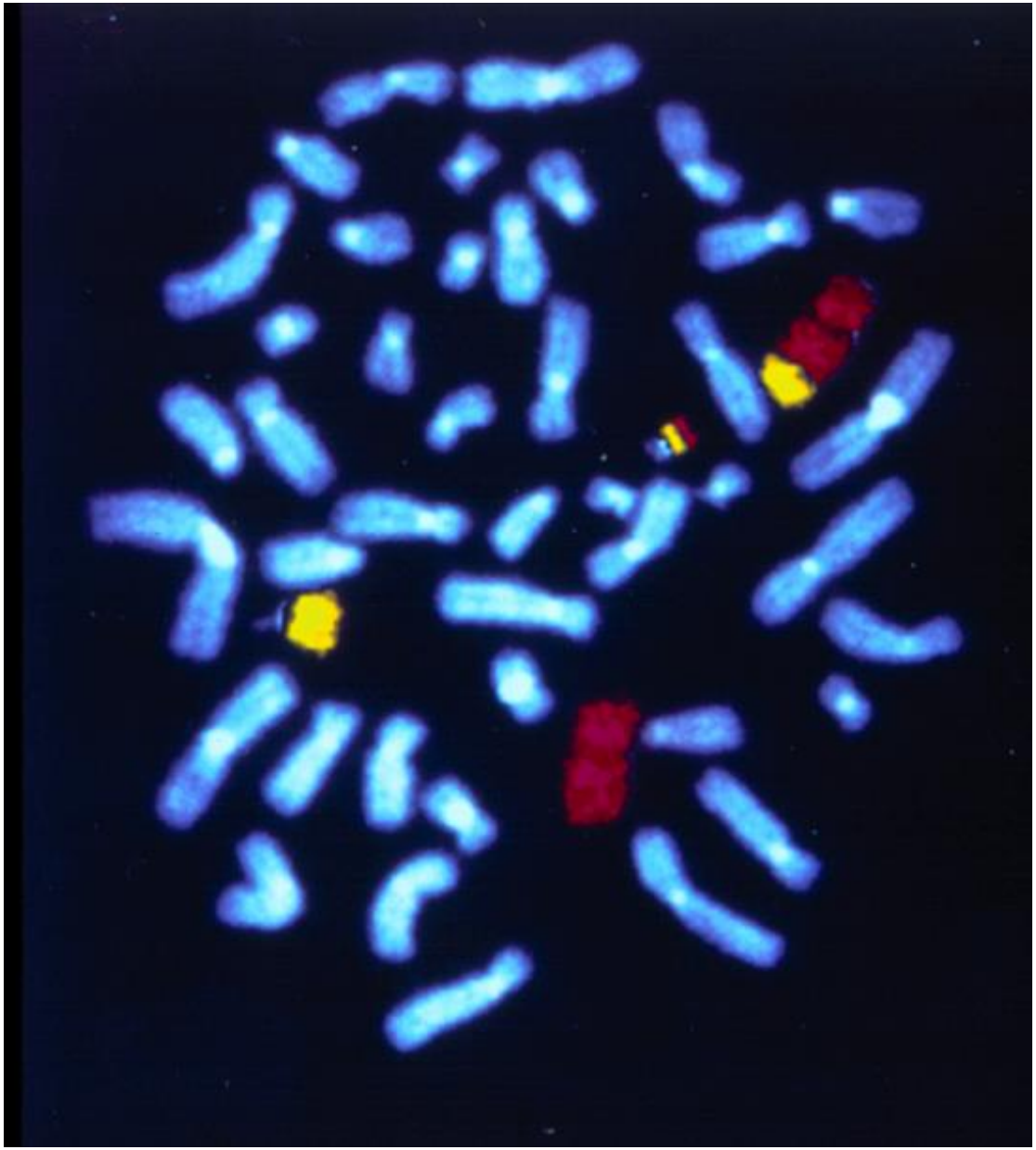
Karyotype examined; normal

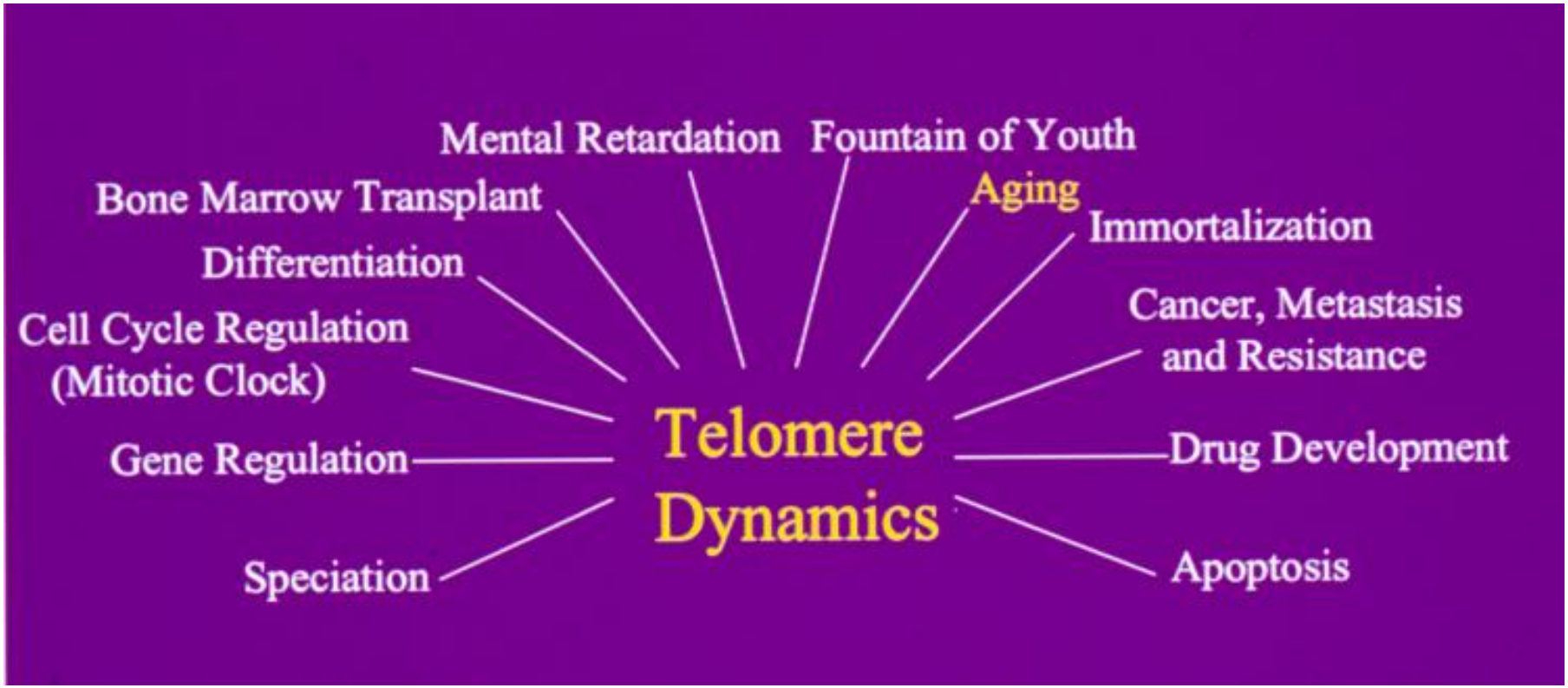
Karyotype not examined

Spontaneous abortion

Person is deceased

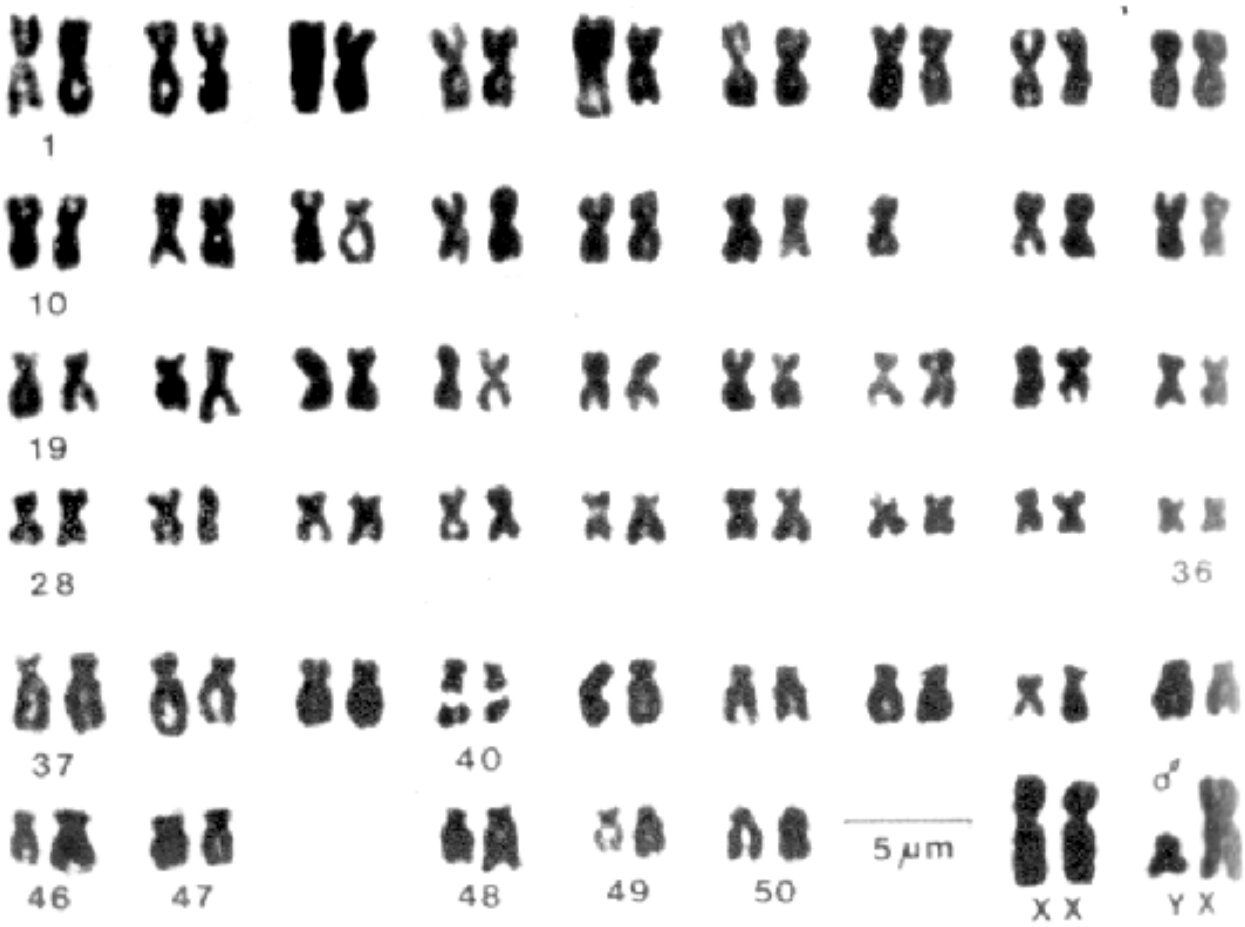





Mental Retardation
Fountain of Youth
Aging
Bone Marrow Transplant
Immortalization
Differentiation
Cancer, Metastasis
and Resistance
Cell Cycle Regulation
(Mitotic Clock)
Telomere
Dynamics
Gene Regulation
Drug Development
Speciation
Apoptosis

Indian Muntjak, 2n = 6♀ , 7♂

Indian Muntjak

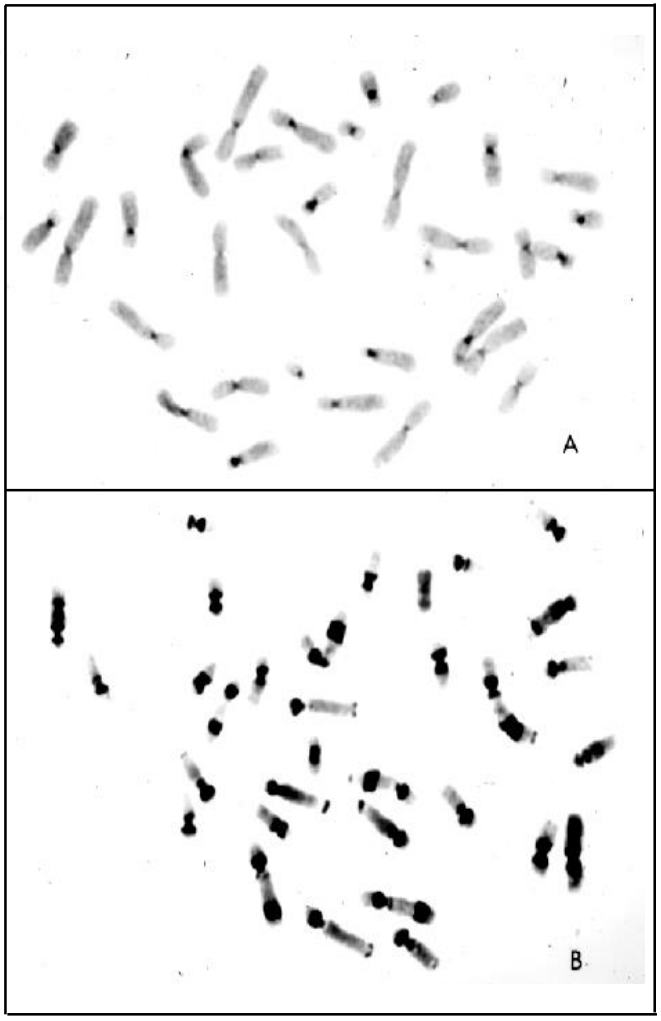


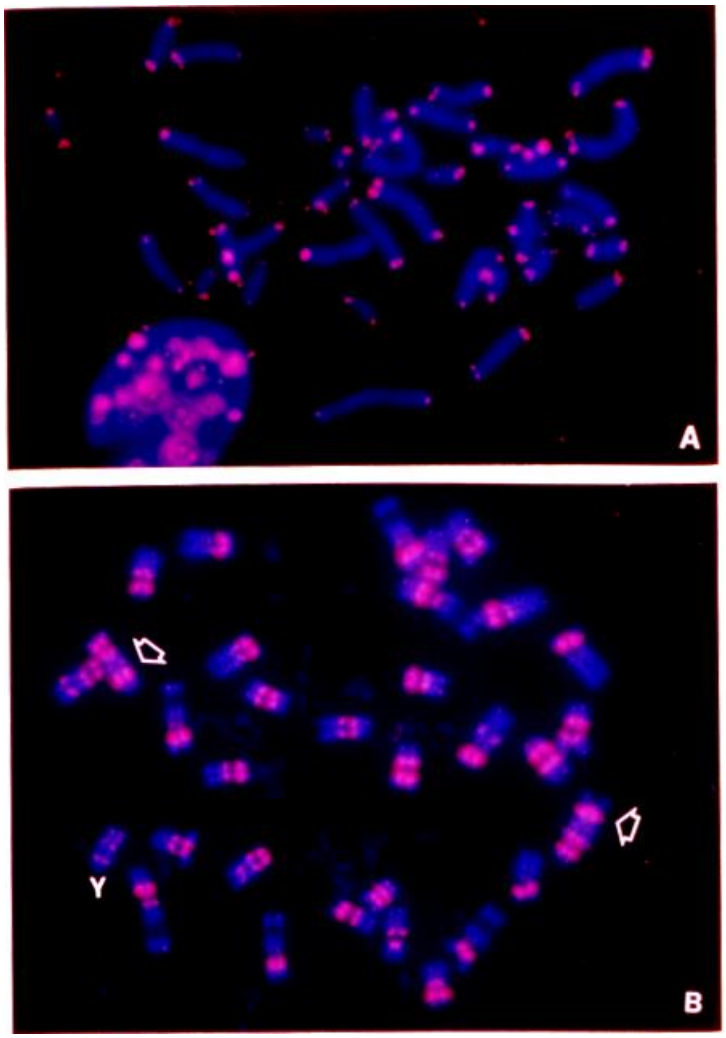
Tympanoctomys barrerae, Viscacha Rat, 2n = 102

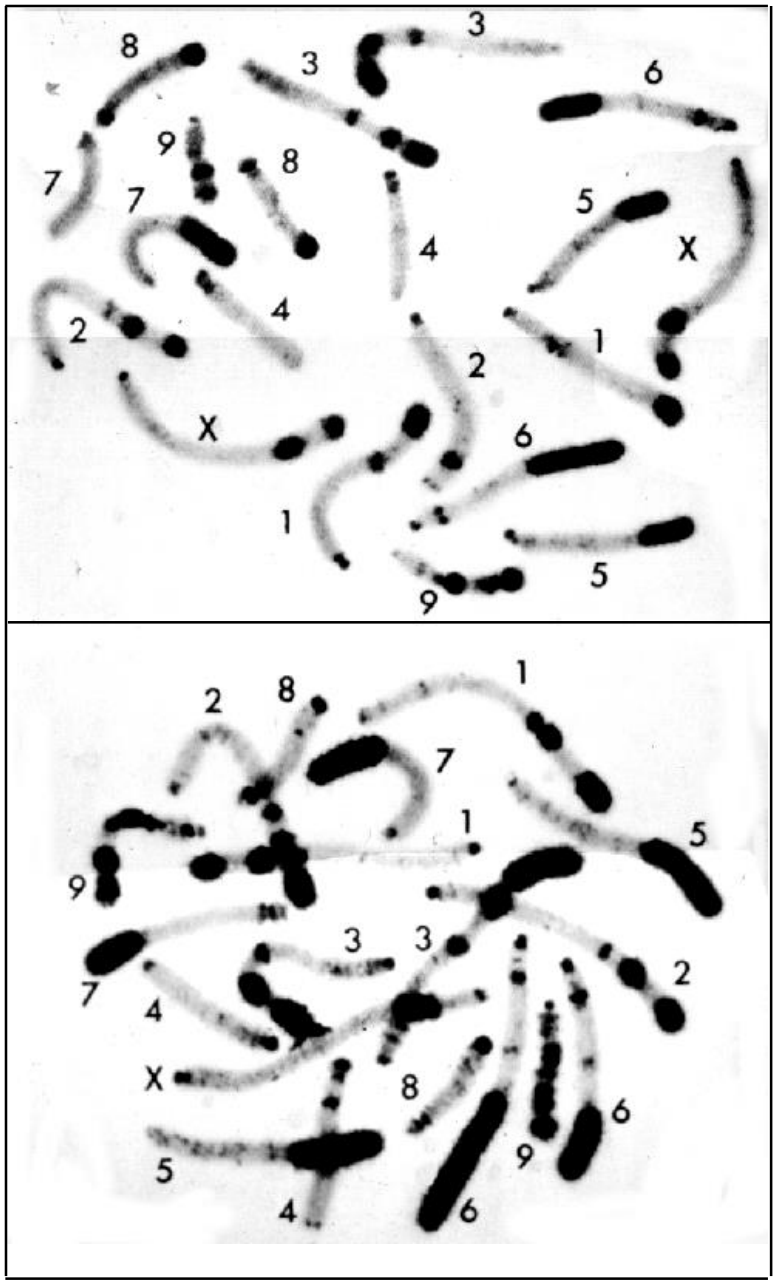
Comparison of Rodentia and Carnivora

Order- Rodentia
Comprises of 35 Families and 443 genera
Family: Muridae
There are 281 genera and 1326 species
(2n = 10 – 102)

Order- Carnivora
Comprises of seven Families and 101 genera
Family: Felidae
There are 17 genera and 38 species
(2n = 36 – 38)


A
B

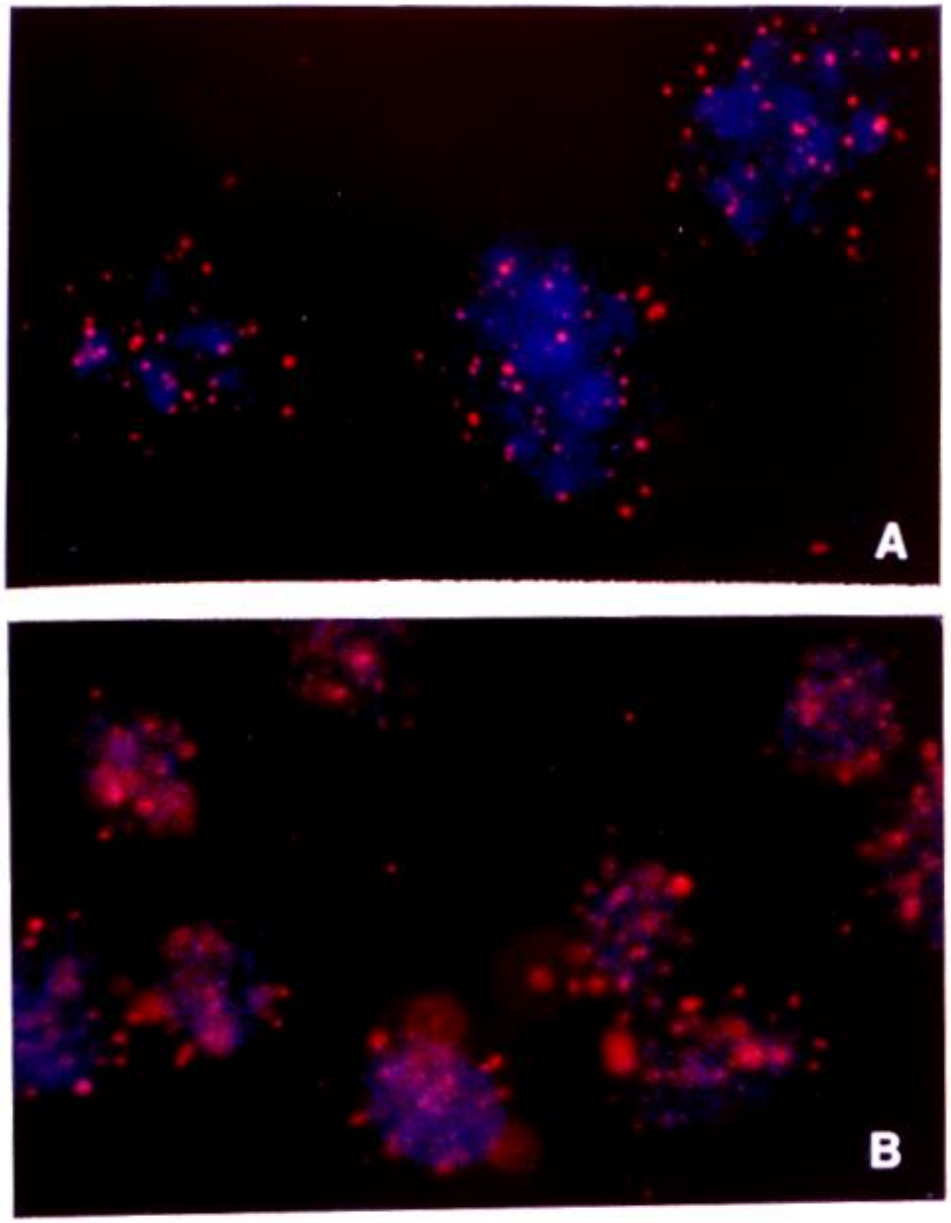

A
Y
B

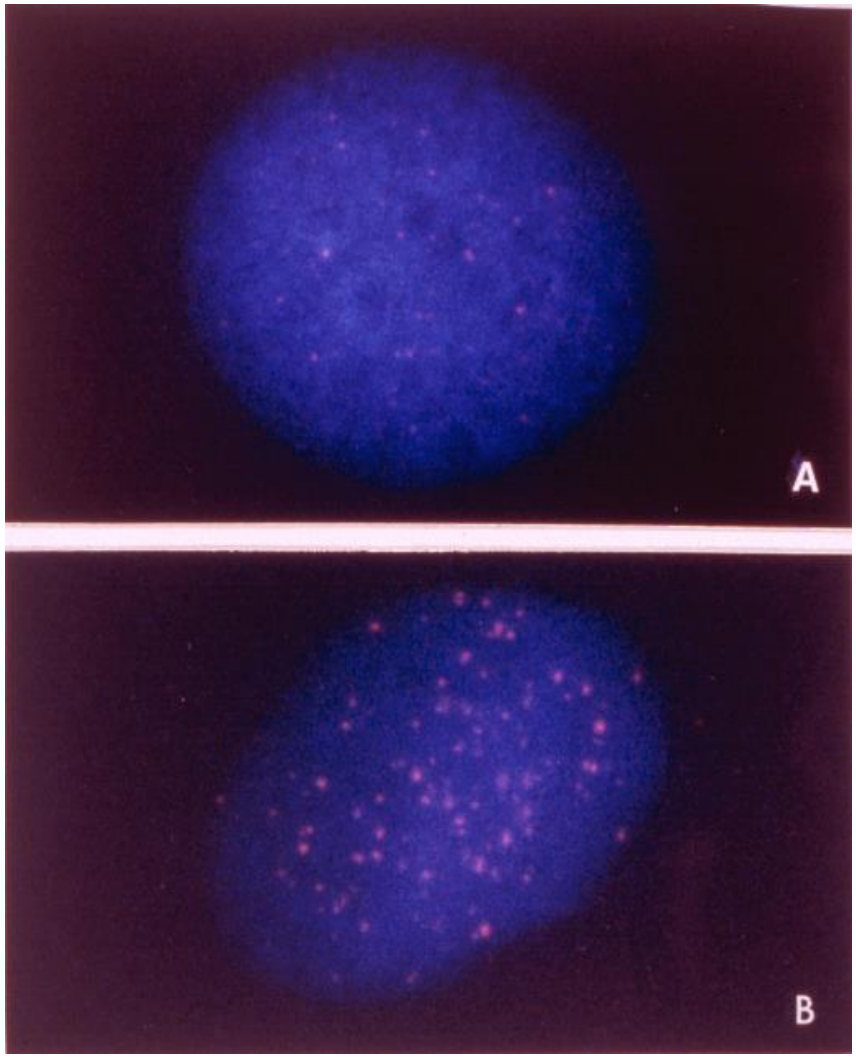


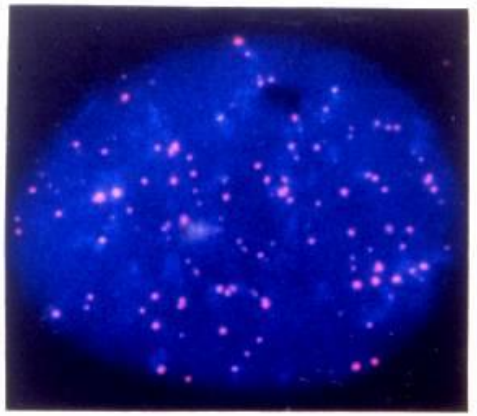
Grasshoppers

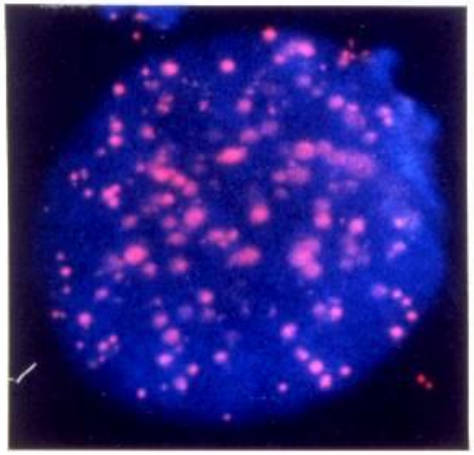
Hypothesis

- **Telomere amplification is a biomarker of cancer metastasis and therapy.**
- **Telomere erosion plays an important role in cell death.**


A
B




A


B

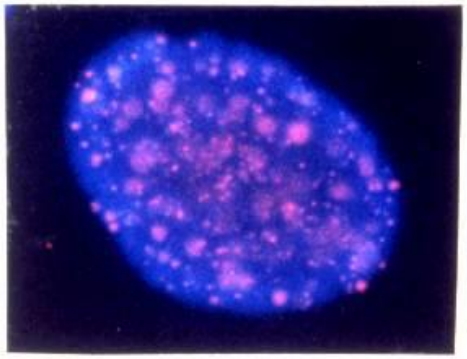

C

Table 1-HUMAN AND MURINE CANCER CELL LINES STUDIED FOR TELOMERIC SIGNAL INTENSITY

Cell Lines	Metastatic or invasive potential	Modal chromosome number	Telomeric area (%)	
			median	mean
Prostate				
LNCaP	N	89	0.05	0.06
C4-2	M	82	1.76	1.80
Breast				
MDA MB 468	N	59	0.29	0.43
MDA MB 435	M	58	2.36	2.82
MDA MB 435 Br.1	LM	56	1.34	1.58
MDA MB 435 lung 2	M	56	2.50	4.03
Renal				
SN12C	LM	110	3.20	2.55
SNPM6	M	60	0.60	0.69
Colon				
KM 12C	LM	83	0.00	0.02
KM 12SM	M	82	0.35	0.40
KM 12L4	M	81	0.11	0.30
Malignant Glioma				
MGR 2	LI	64	0.22	0.29
HBT 5	LI	68	0.05	0.06
MGR1	HI	63	0.80	1.70

N = Nonmetastatic; LM = lowly metastatic; M = metastatic; LI = low or non-invasive; HI = highly invasive

Table I - Continued

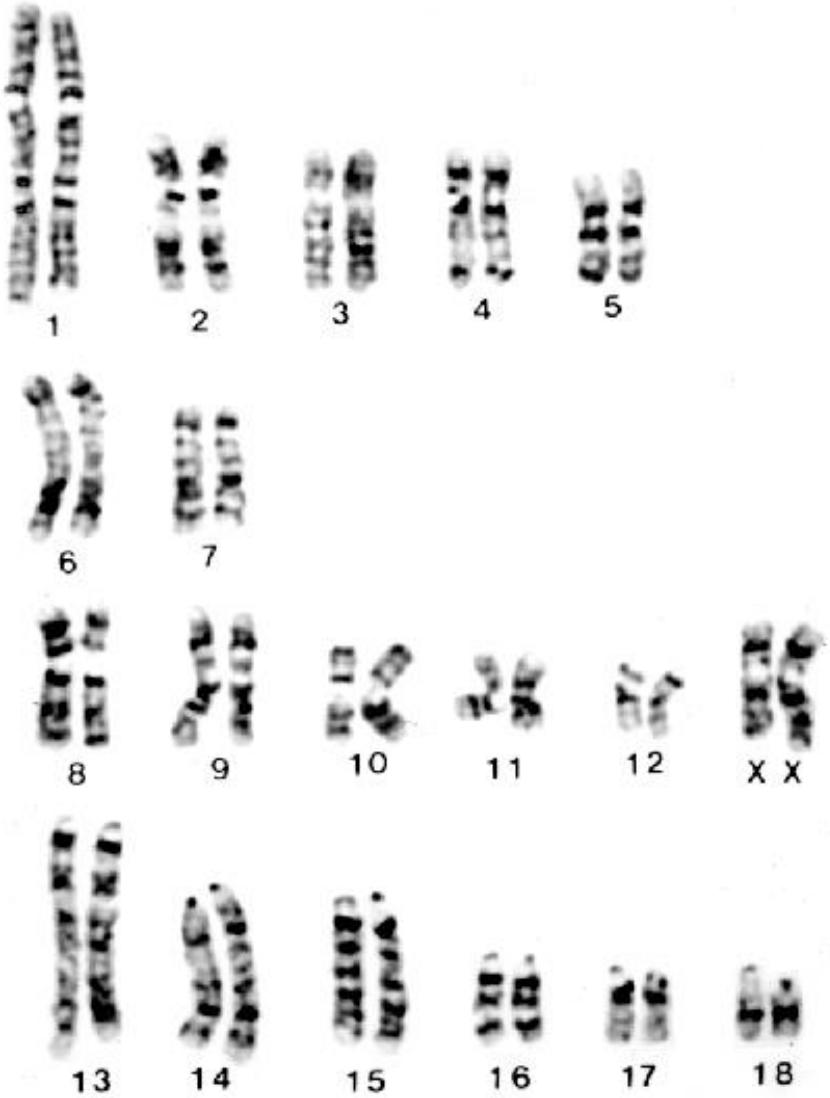
Cell Lines	Metastatic or invasive potential	Modal chromosome number	Telomeric area (%)	
			median	mean
Human melanoma				
A375P	LM	125	0.73	0.92
A375SM	M	81	1.61	2.04
A375C15N	M	64	1.09	1.39
TXM-18	LM	70	0.00	0.03
TXM-1	M	68	0.39	0.42
Mouse Melanoma				
K1735P	LM	45	6.96	8.37
K1735 C23	N	42	2.40	2.49
K1735 C4	M	44	6.42	7.31
K1735 CX21	M	41	5.90	7.80
Human-mouse melanoma hybrid				
H17(A375C18N x K1735C19H)	LM	110	0.82	0.88
H6(A375C15N x K1735C19H)	M	151	2.79	5.03
H1/SM(A375C18N x K1735C3H)	M	131	4.41	4.54
H34/MEM(A375C18N x K1735C3H)	M	107	3.05	3.73

N = Nonmetastatic; LM = lowly metastatic; M = metastatic; LI = low or non-invasive; HI = highly invasive

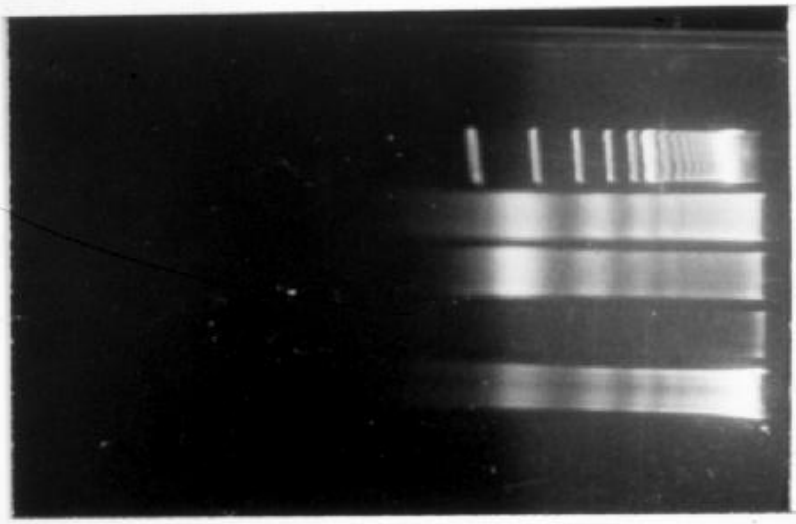
Table 1: Telomere studies in PHA-stimulated lymphocytes of prostate cancer patients

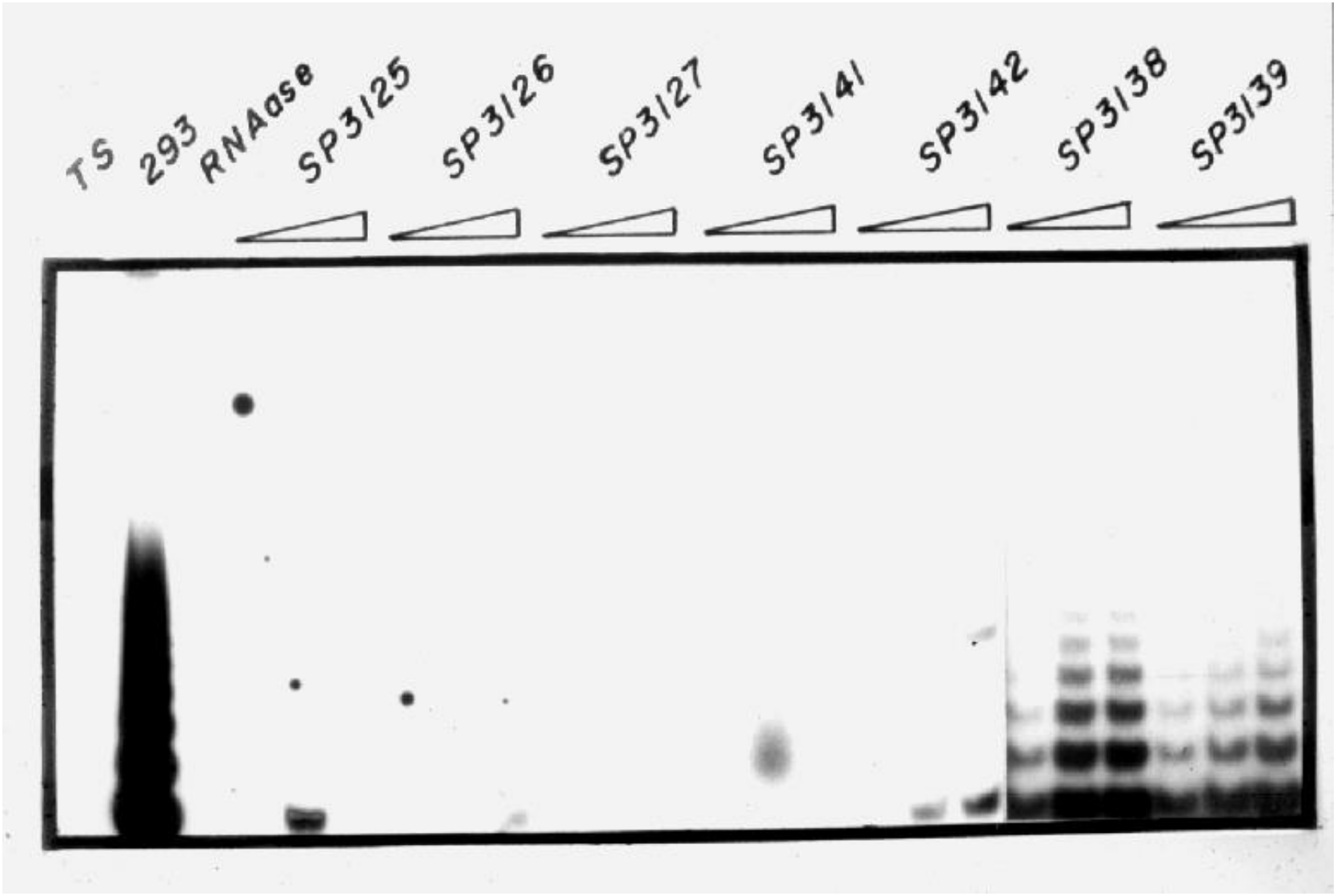
No.	Category	Our lab. No.	Age (yr)	Ethni-city	G.G	PSA Levels (ng/mL)	% telomeric area
1	C	HB 1880	46	AA			1.61
2	C	HB 1881	68	AA			1.69
3	C	HB 1882	47	AA			1.73
4	C	HB 1883	47	AA			1.42
5	C	HB 1884	49	AA			2.03
6	C	HB 1885	58	AA			1.45
7	C	HB 1886	53	AA			1.59
8	C	HB 1887	56	AA			1.70
9	C	HB 1888	58	AA			1.64
1	NM	HB 1690	59	W			1.81
2	NM	HB 1653	53	W	6		2.13
3	NM	HB 1669	51	W			1.00
4	NM	HB 1708	47	W	7		1.04
5	NM	HB 1683	61	W	3+4	4.9	1.47
6	NM	HB 1631	55	AA	7	UD	1.14
7	NM	HB 1637	76	W	8	<0.2	1.82
8	NM	HB 1629	61	W	8	14.2	1.65
9	NM	HB 1623	67	AA	7	7.6	1.40
10	NM	HB 1645	60	W	7	5.0	0.92
11	NM	HB 1614	51	W	7	UD	1.70
12	NM	HB 1682	65	H			1.38
13	NM	HB 1624	54	W	8	UD	1.33
14	NM	HB 1654	70	W	9		1.04
15	NM	HB 1704	64	AA		UD	1.98
1	M	HB 1729	68	H		281	2.47
2	M	HB 1620	44	W			2.57
3	M	HB 1720	50	H			3.05
4	M	HB 1689		W			3.03
5	M	HB 1681	61	W		40.0	2.53
6	M	HB 1673	65	W			2.40
7	M	HB 1670	78	W			3.35
8	M	HB 1630	69	W		0.3	2.37
9	M	HB 1646	59	W		0.2	1.76
10	M	HB 1647	67	H		13.4	3.08
11	M	HB 1746	55	W		<0.1	3.27

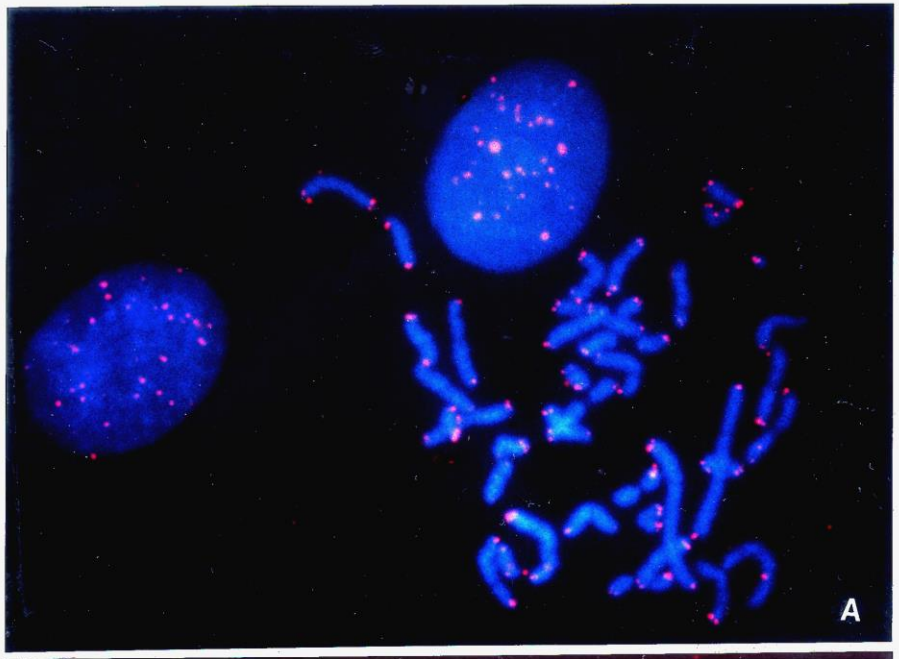
C=controls; NM= Nonmetastatic; M= Metastatic disease G G=Gleason grade
W= White; H= Hispanic; AA= African American
UD- undetectable

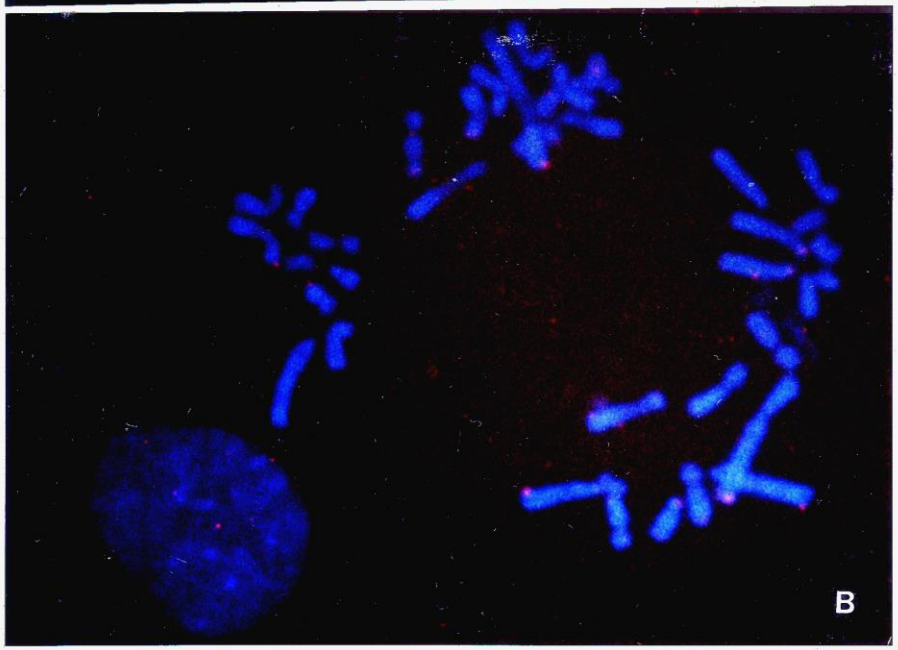

1
2
3
4
5
6
7
8
9
10
11
12
X X
13
14
15
16
17
18

A
B
C

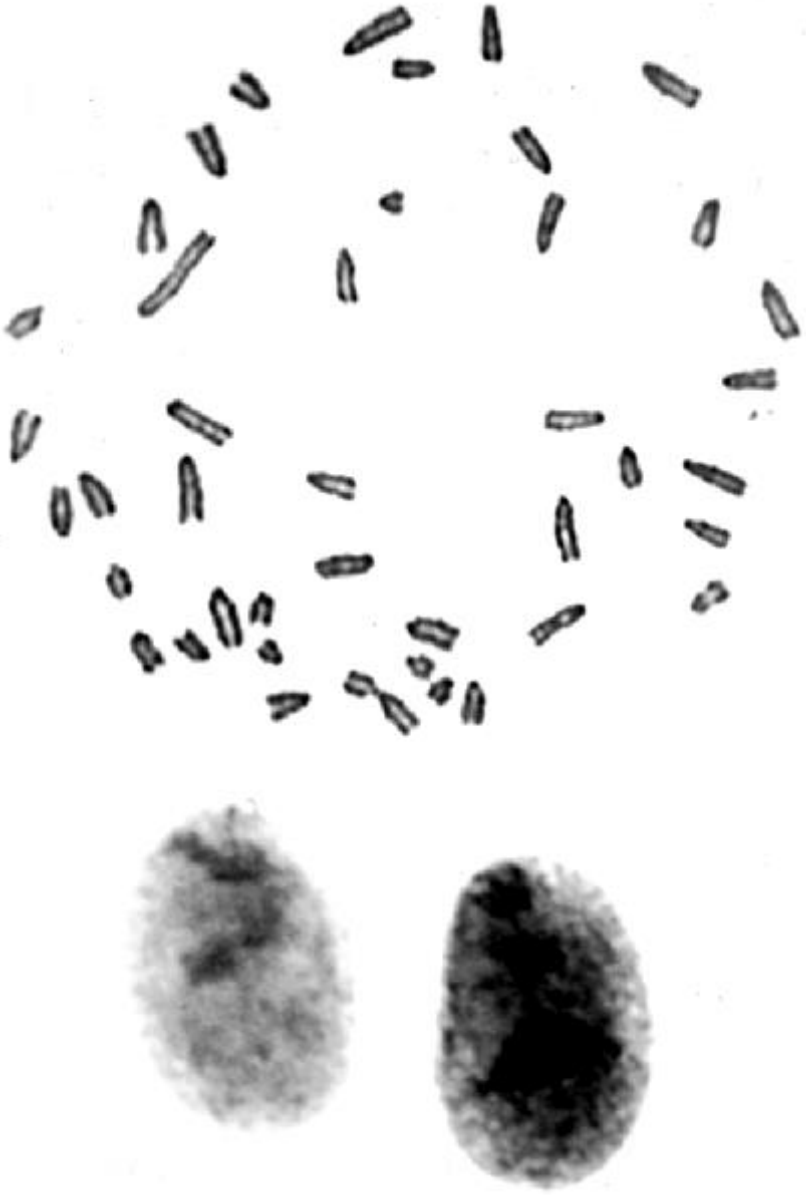

100 bp ladder
TU 8315
TU8316
TU8317
TU8318

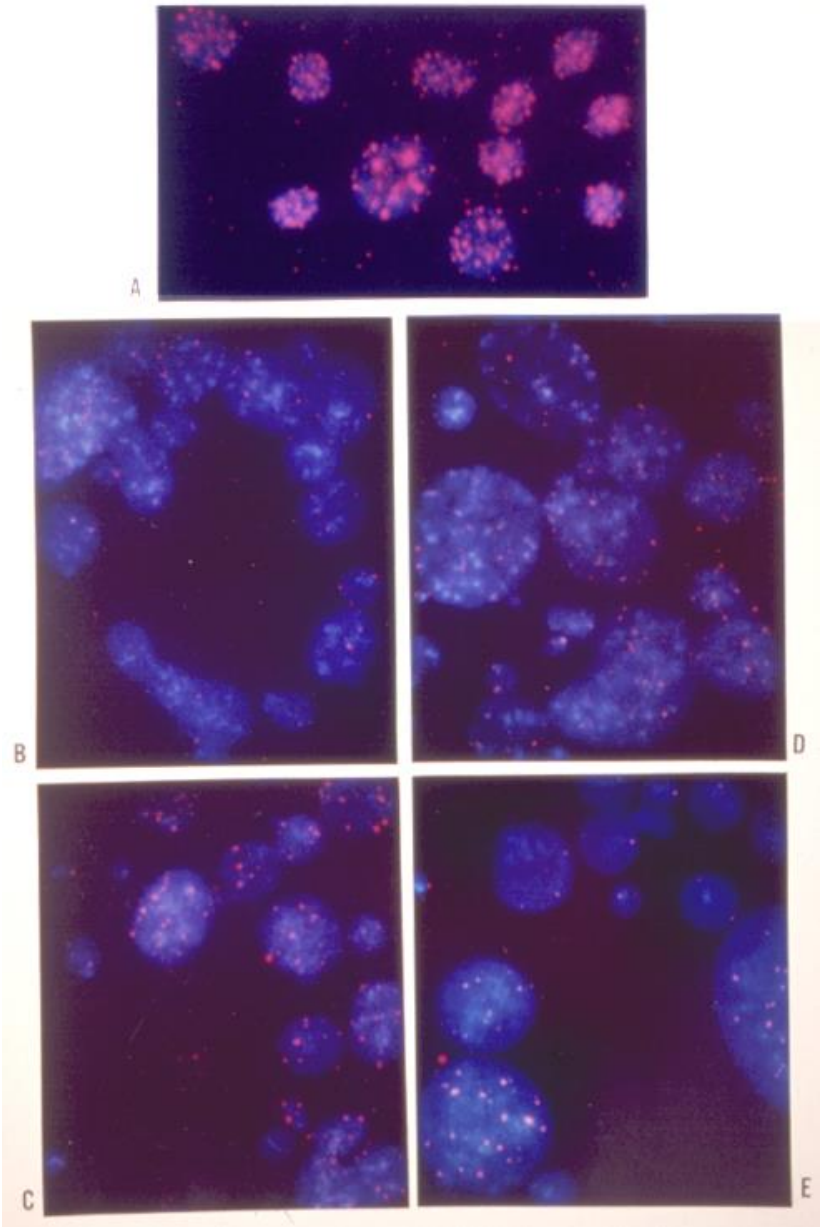

TS
293
RNAase
SP3125
SP3126
SP3127
SP3141
SP3142
SP3138
SP3139

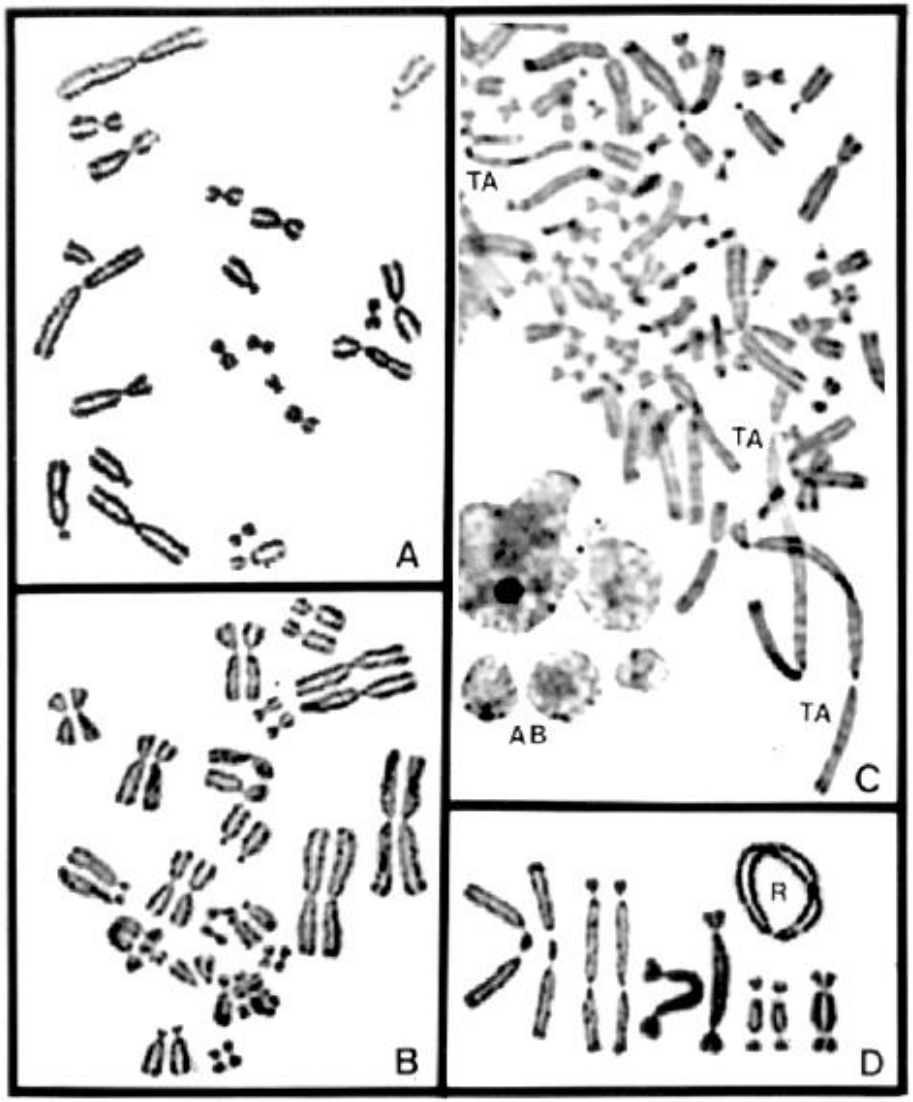

A


B

Paclitaxel-treated K1735-X-21

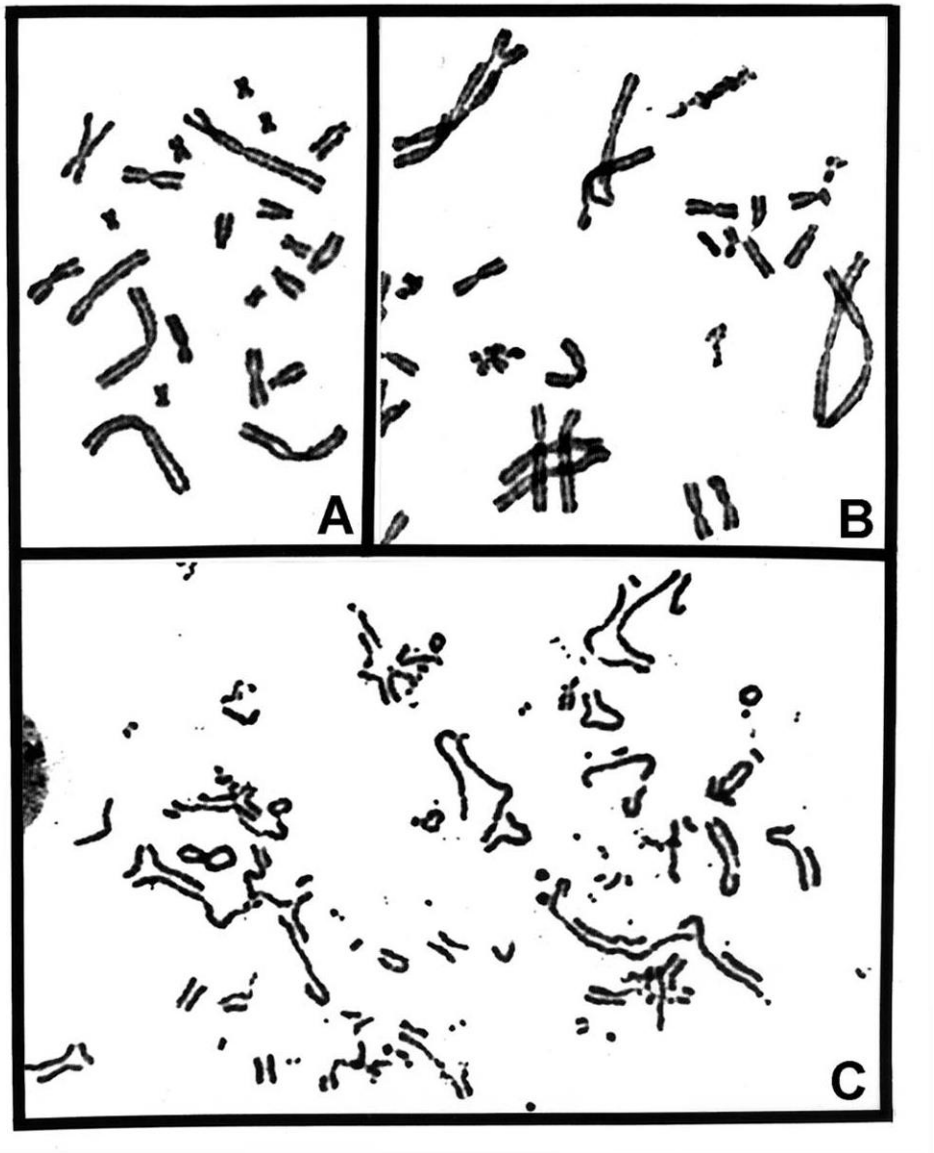



A
B
D
C
E

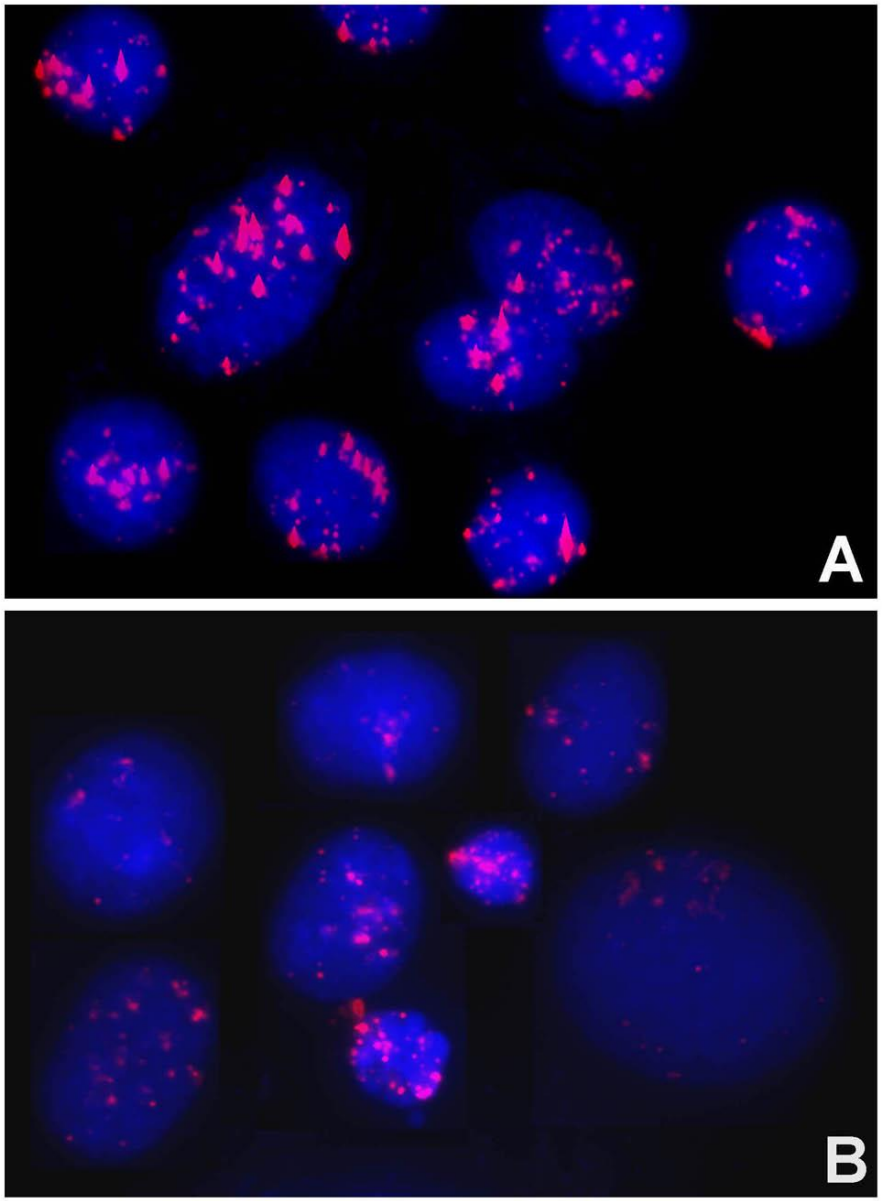


Multani et al., (Mutant CHO), 1999.

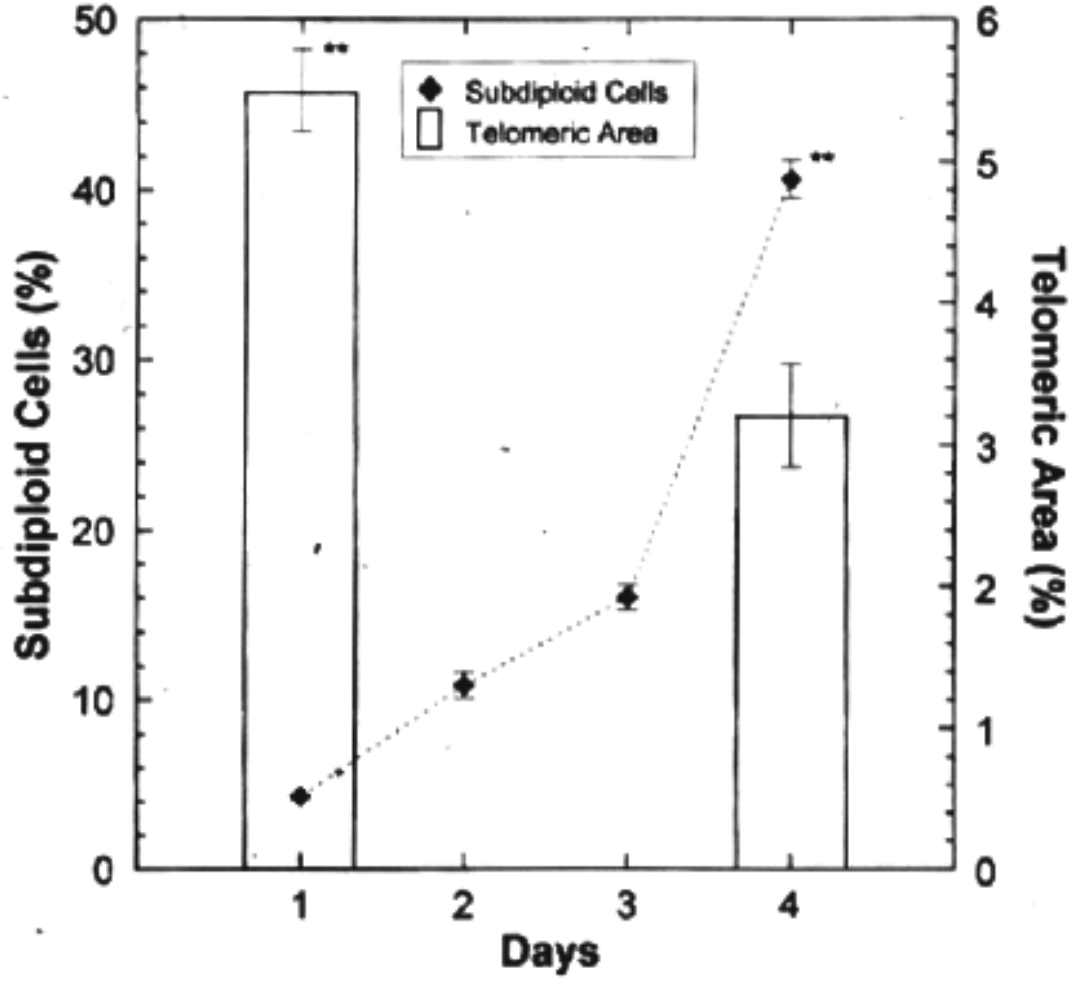
Metaphases from aging DON cells

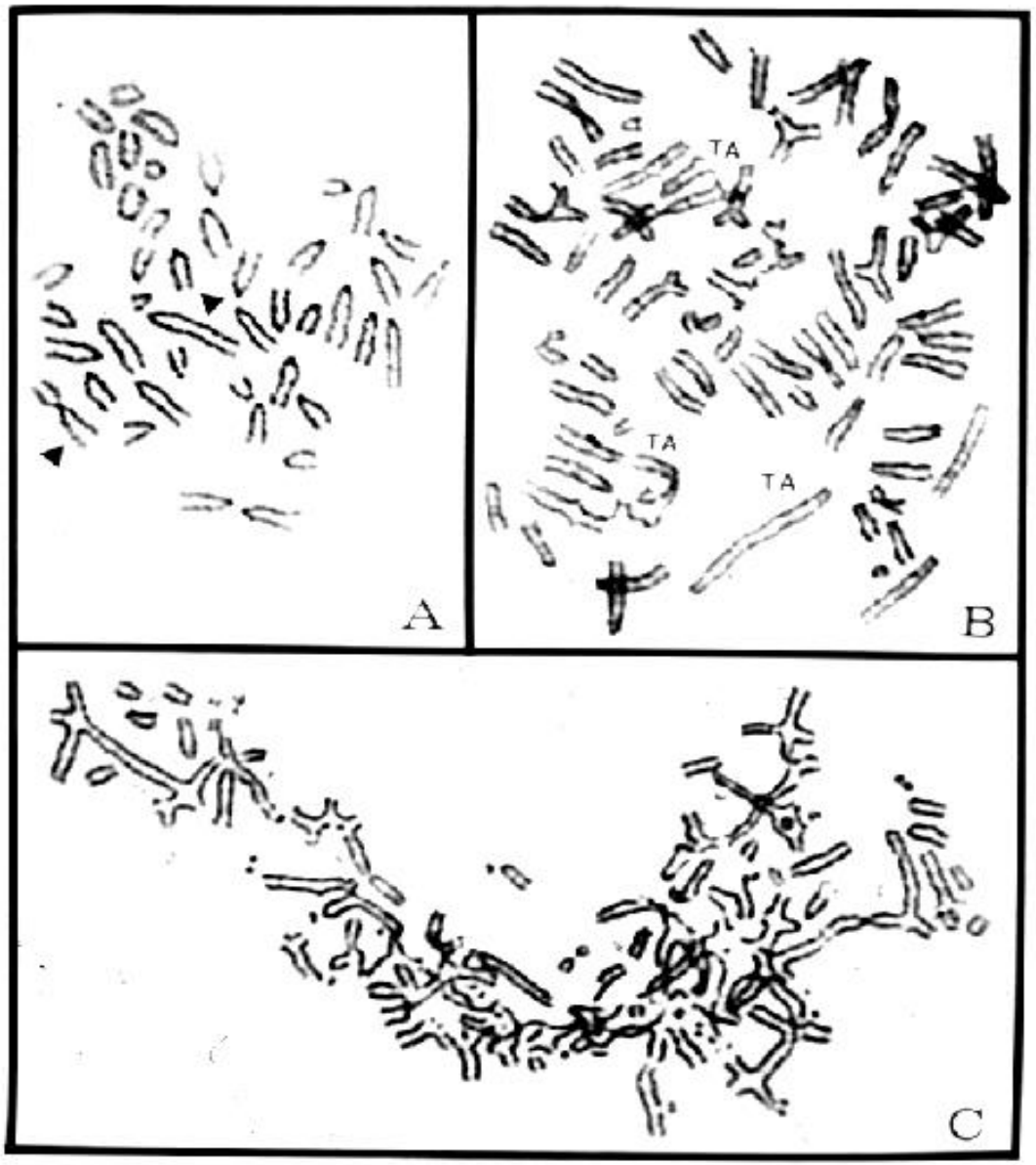


Senescent DON cells

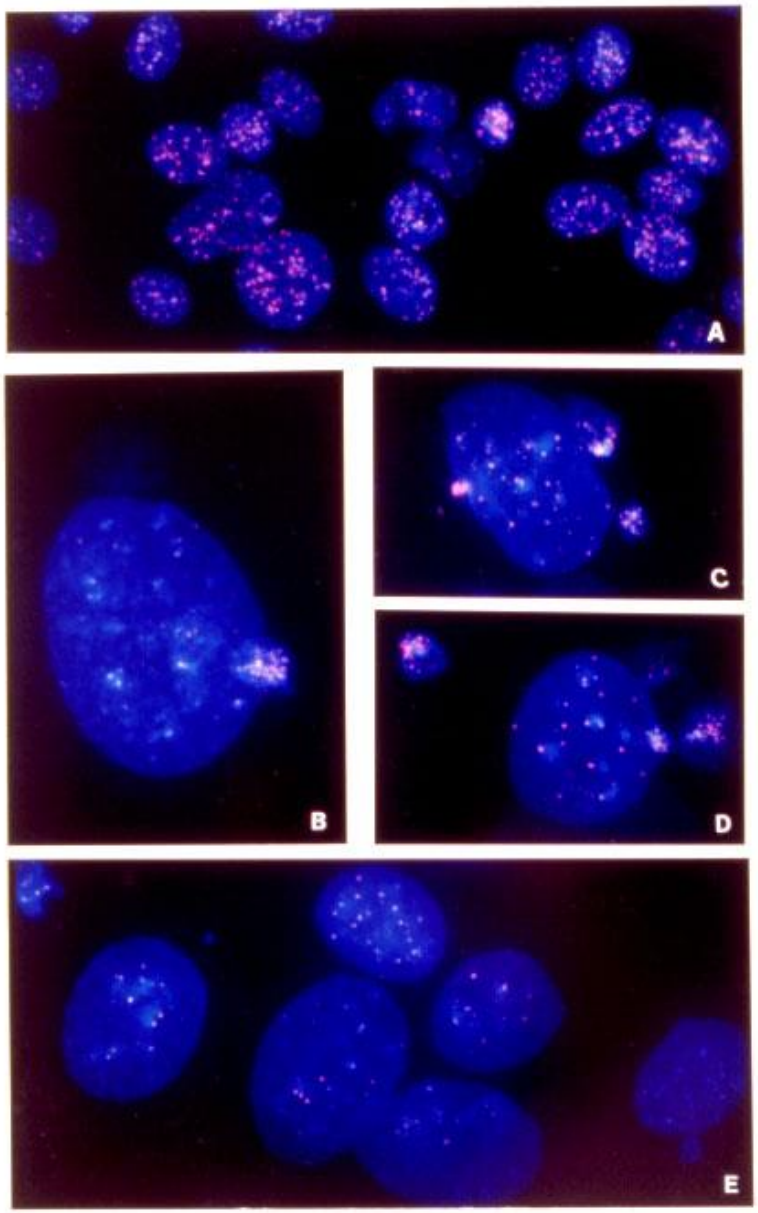


Senescent Don cells

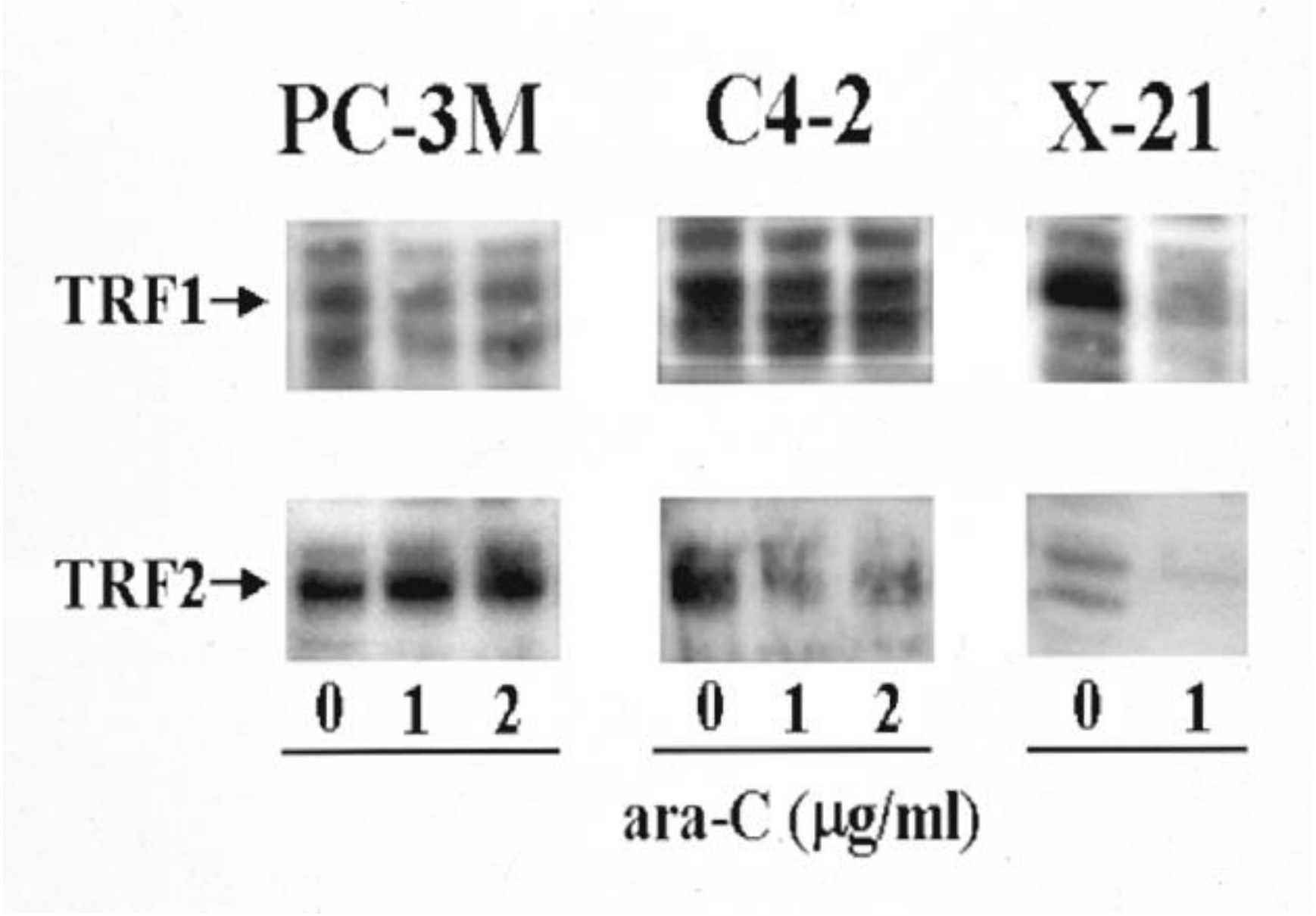




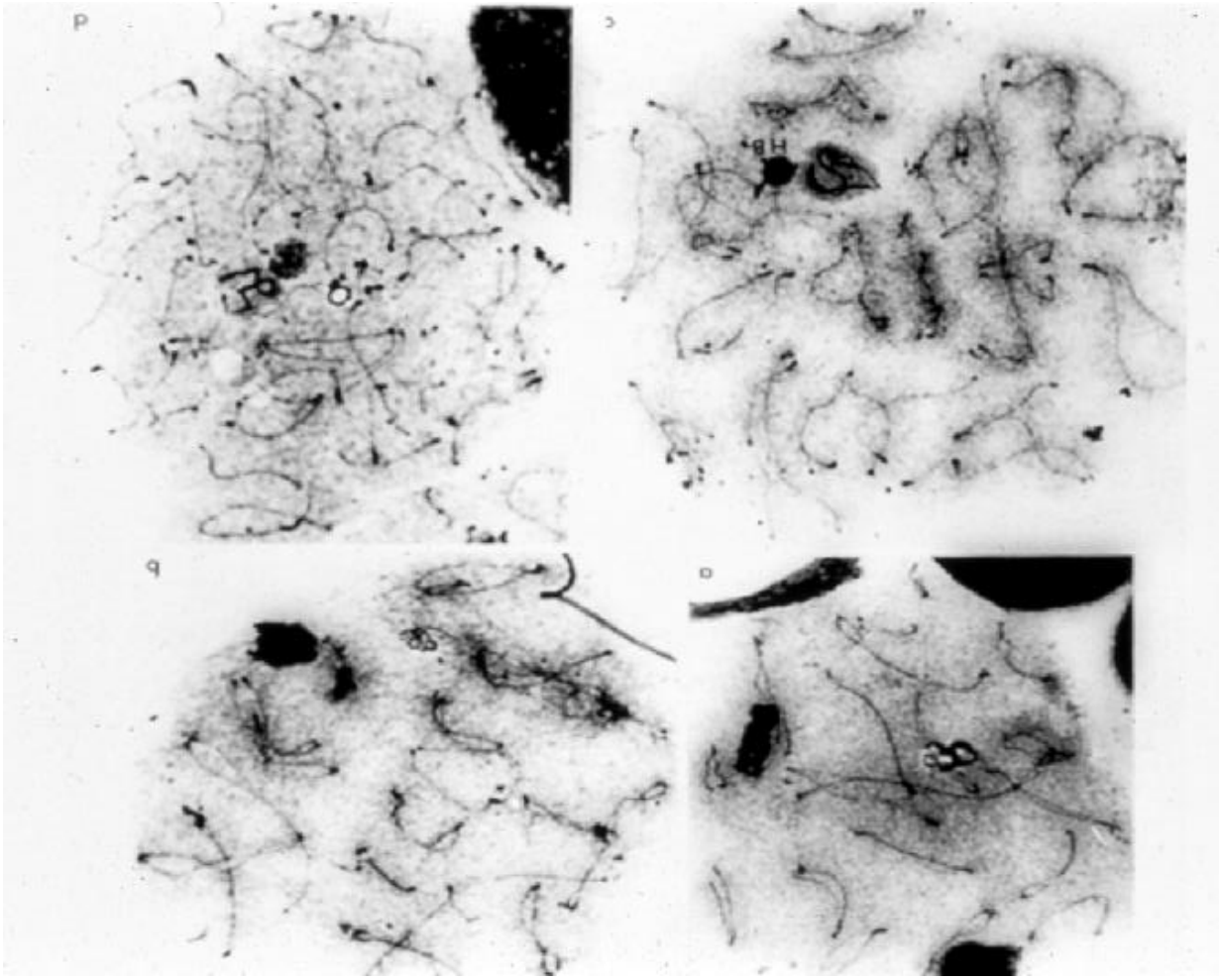
Ara-C –treated X-21 clone


A
B
C
D
E

Western blot




NEOPLASIA
An International Journal for Oncology Research
Volume 2 Number 4
July/August 2000
Telomere Degradation in Apoptosis
DCC Alterations in Lung Cancer
The Shared Machinery of Cell Life and Death
Adaptation to Anti-Angiogenic Therapy
Now on MEDLINE and Current Contents

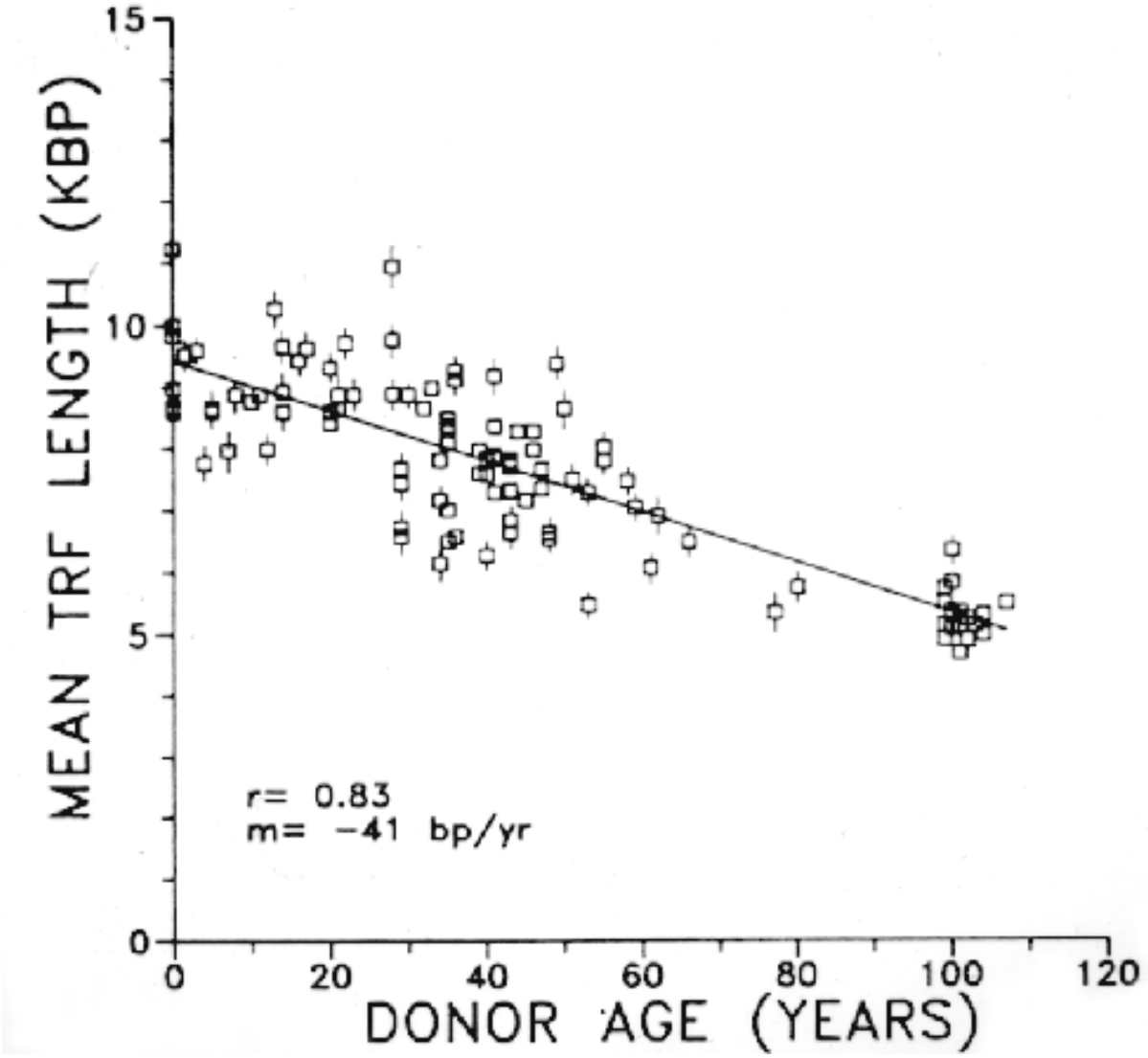

p
c
q
d

Conclusions

- Telomere reduction is the earliest event for cell death (spontaneous or induced)
- Telomere amplification is the molecular clock for metastasis and drug resistance

Syndromes with accelerated aging

- Progeria
- Werner Syndrome
- Ataxia Telangiectasia (AT)
- Down Syndrome


MEAN TRF LENGTH (KBP)
15
10
5
0
0
20
40
60
80
100
120
DONOR AGE (YEARS)
r= 0.83
m= −41 bp/yr

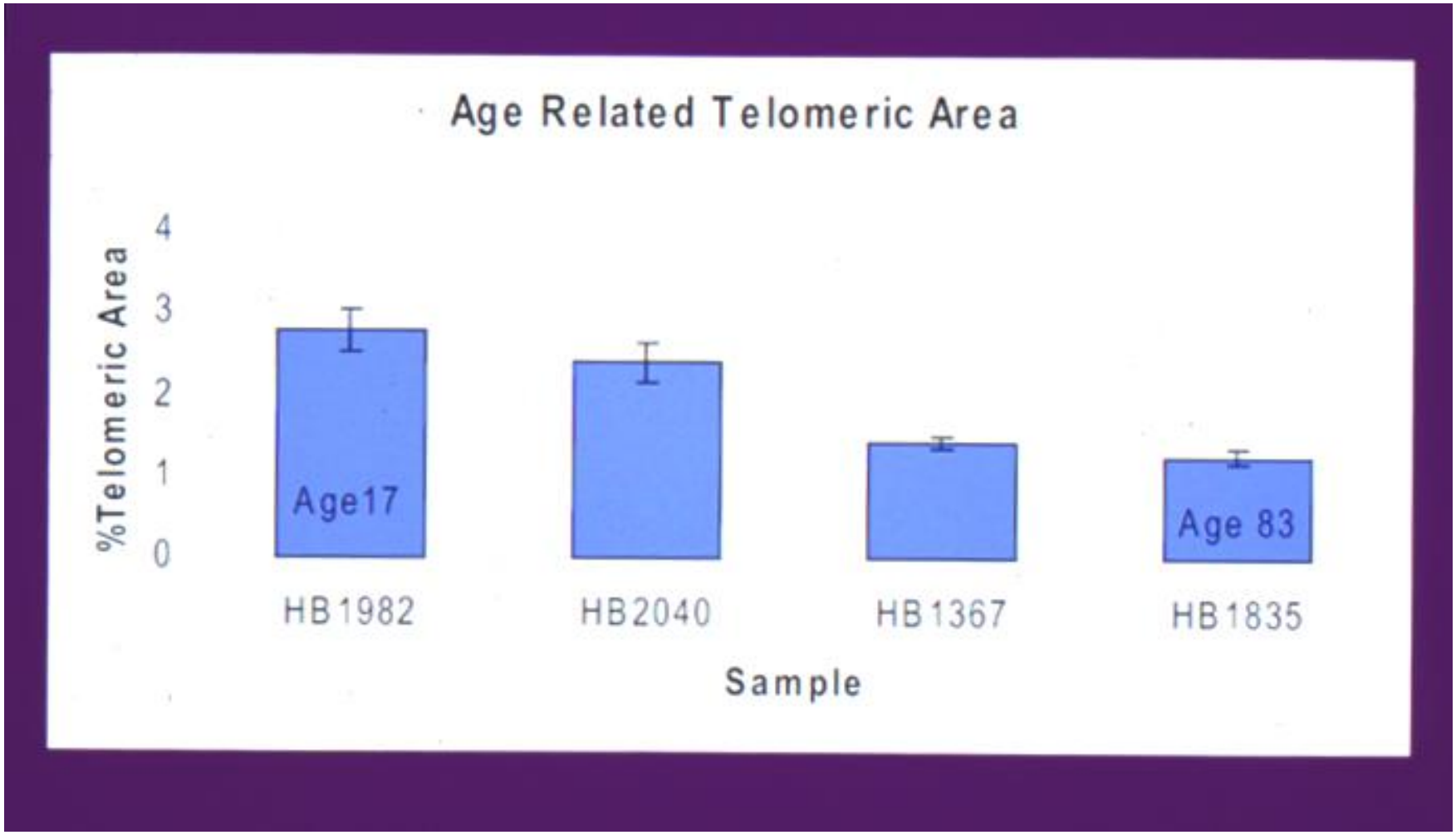
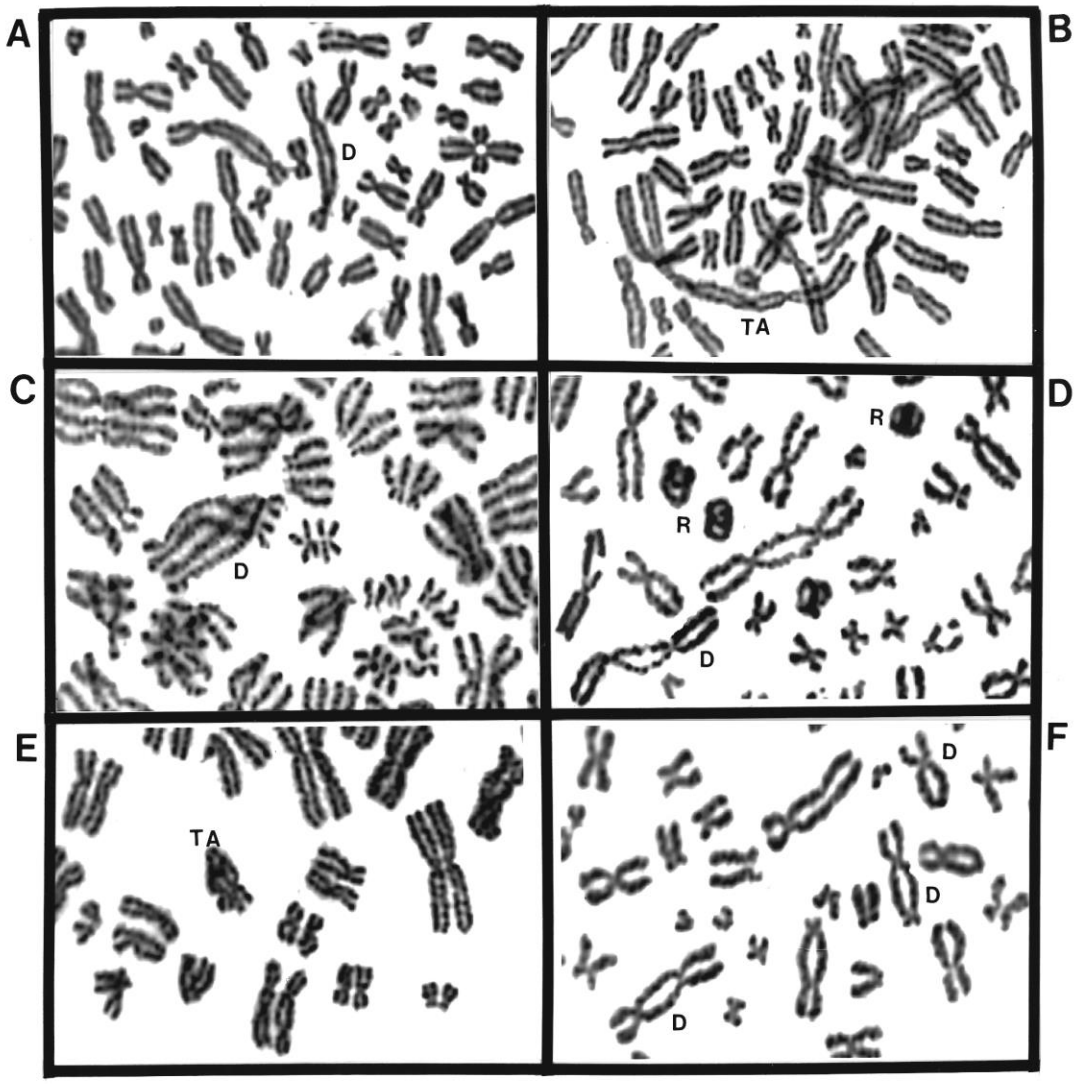
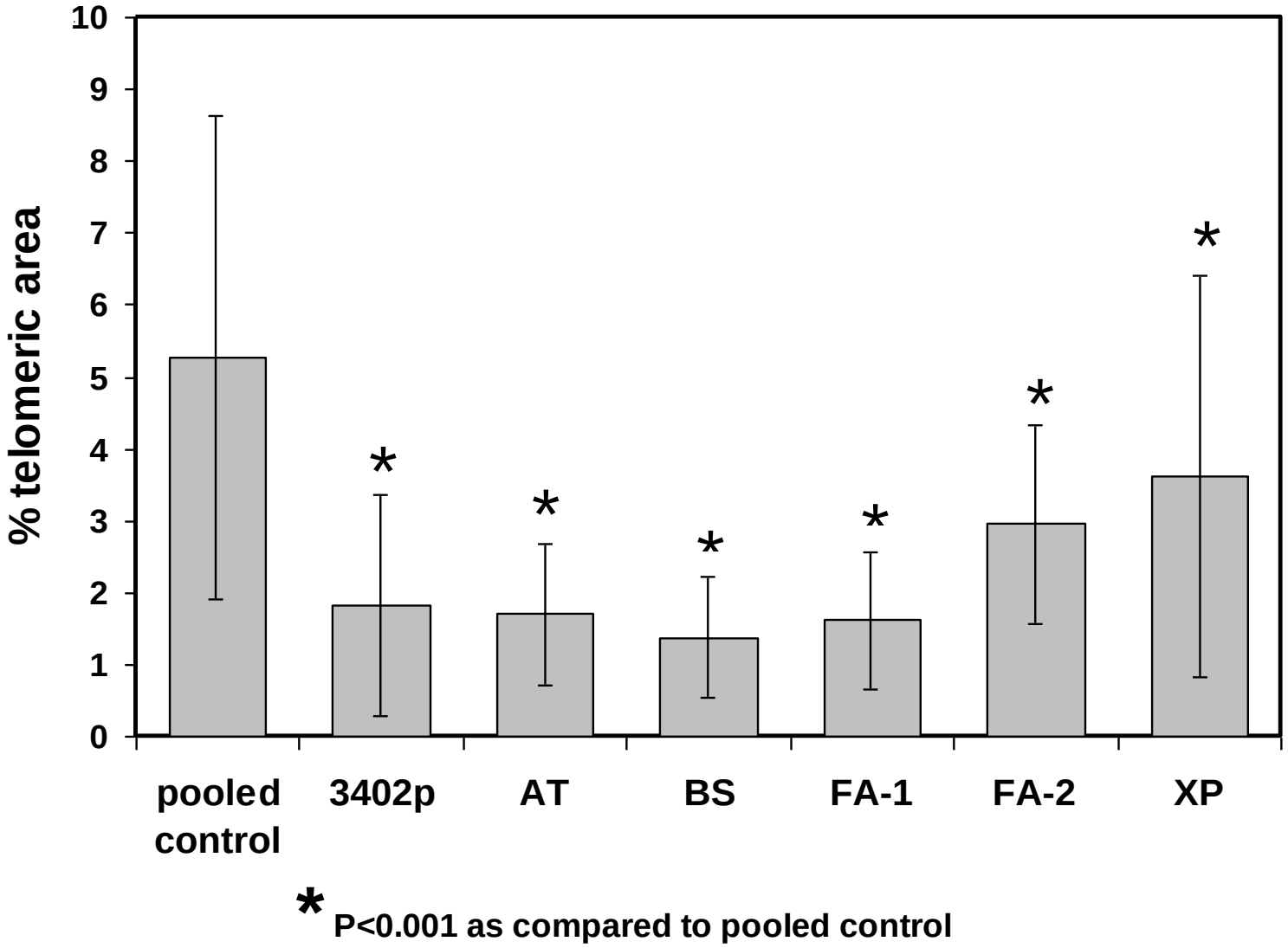

Age Related Telomeric Area
%Telomeric Area
4
3
2
1
0
Age17
Age 83
HB1982
HB2040
HB1367
HB1835
Sample

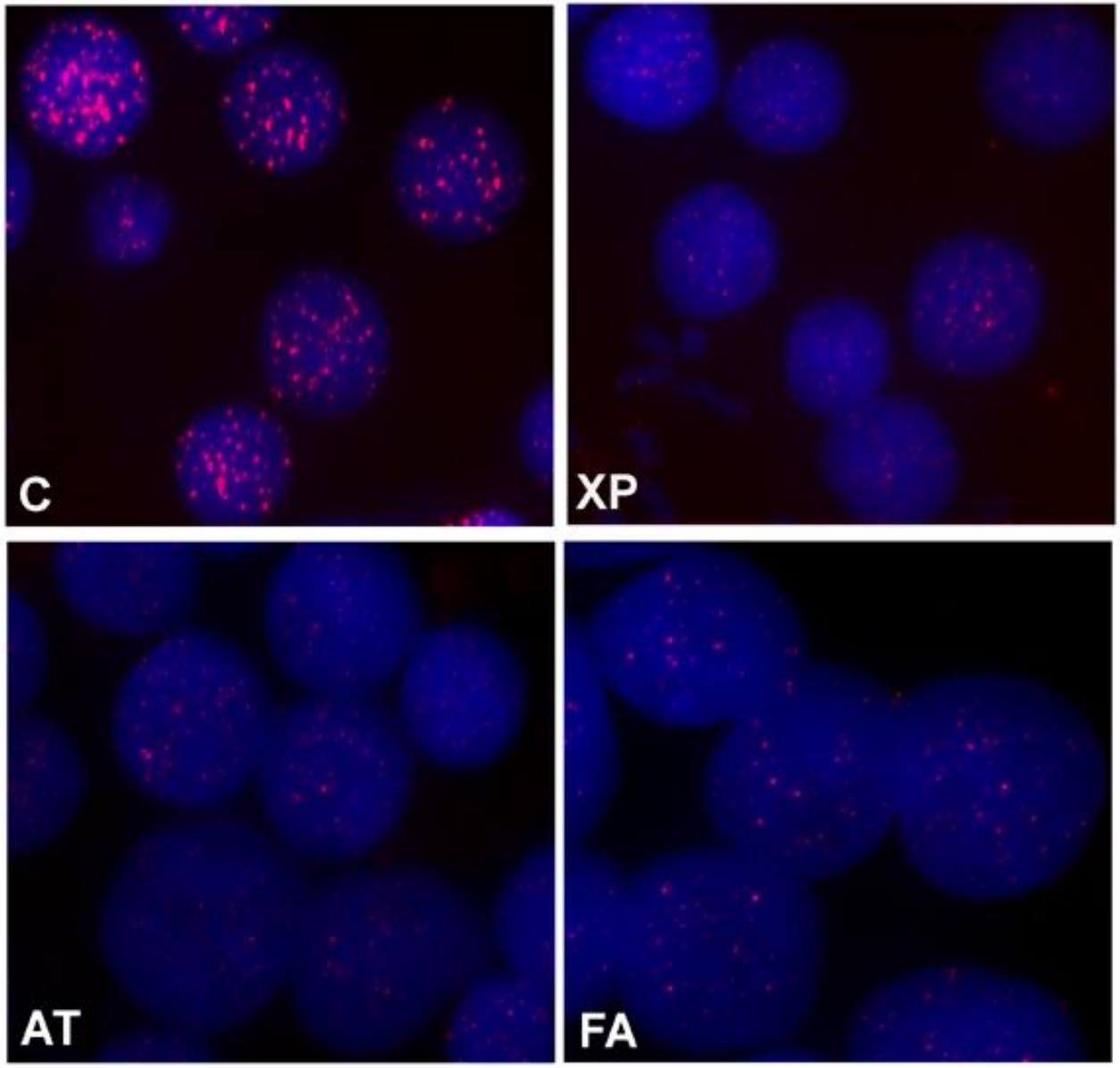
Table 1. Frequency of abnormal metaphases in B-lymphoblastoid cells of the chromosome-breakage syndromes and normals.

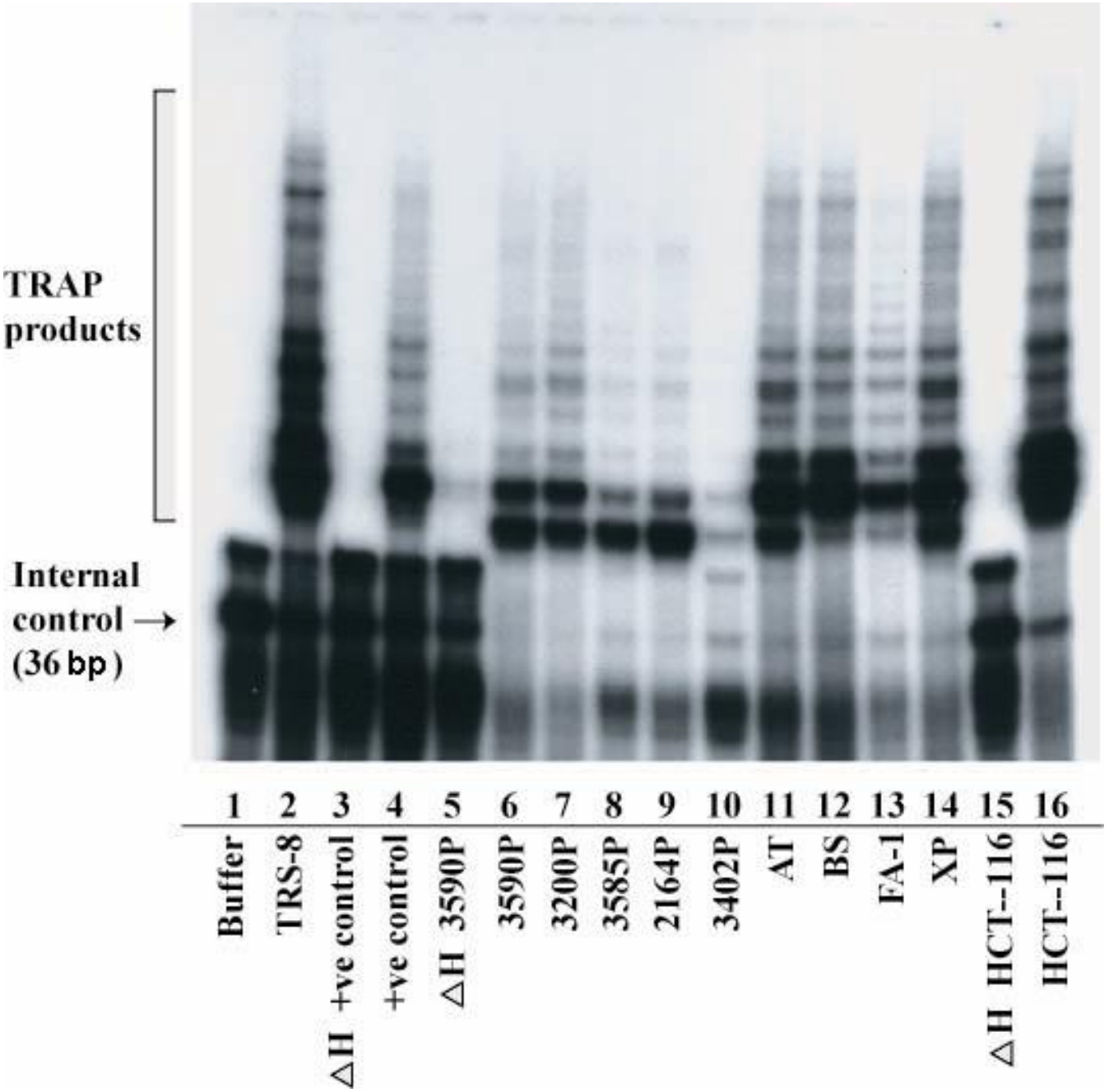
B-cell lines	Normal metaphases (%)	Endoreduplication (%)	Tetraploidy (%)	Total (%)	No. of metaphases examined	Metaphases with aberrations (%)
XP	75.7	9.5	14.9	24.4	222	1.4
FA-1	79.3	4.3	16.5	20.8	188	4.8
FA-2	86.3	1.0	7.8	8.8	102	4.9
BS	74.1	5.8	12.7	25.8	205	7.8
AT	79.1	6.8	7.2	20.9	249	6.8
Controls						
3402P*	87.4	2.2	7.0	12.7	229	3.5
3200P	94.7	0.6	5.4	6.0	166	0.6
3590P	95.1	0.0	4.9	4.9	164	0.6
3585P	93.8	0.6	5.6	6.2	177	0.6
2164	90.9	0.0	8.2	8.2	110	0.9

XP = Xeroderma pigmentosum; FA = Fanconi's anemia; BS = Bloom's syndrome; AT = Ataxia-telangiectasia; * = B-cell line from a "normal" female showing higher rate of aberrations.


A
D
B
TA
C
D
D
R
R
D
E
TA
F
D
D
D


% telomeric area
10
9
8
7
6
5
4
3
2
1
0
*
*
*
*
*
*
pooled control
3402p
AT
BS
FA-1
FA-2
XP
* P<0.001 as compared to pooled control


C
XP
AT
FA


TRAP products
Internal control → (36 bp)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Buffer
TRS-8
ΔH +ve control
+ve control
ΔH 3590P
3590P
3200P
3585P
2164P
3402P
AT
BS
FA-1
XP
ΔH HCT--116
HCT--116

Mutagen Sensitive Family

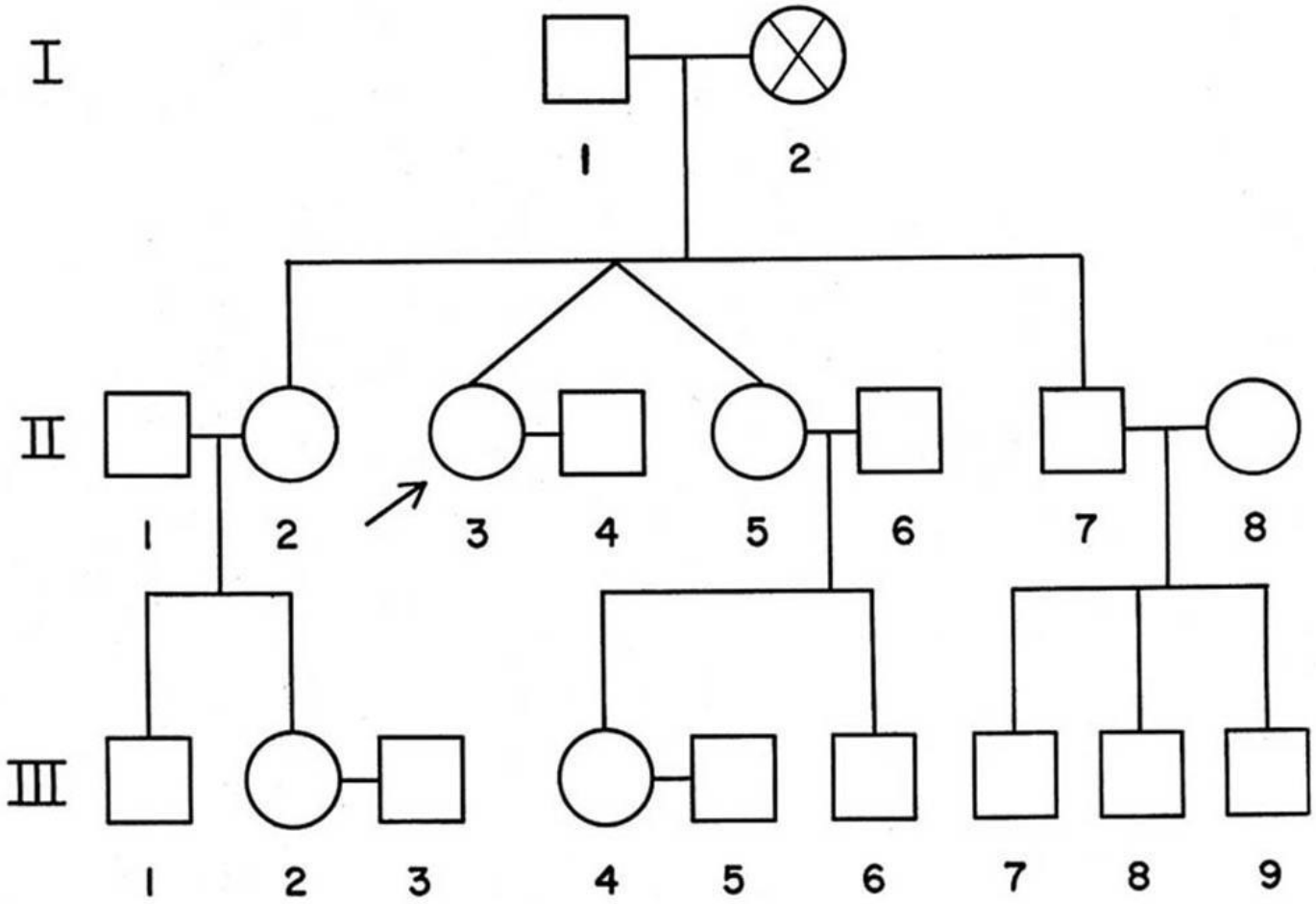


Table I. *Clinical features, sensitivity to clastogens, telomeric area percentage and frequency of TAs in the family members studied.*

Family members	Sex/age (years)	Complexion/ eye color/hair color	Metaphases with TAs (%)	TAs per cell	Sensitivity b/c		Telomeric area %; (mean ± SE[a])
					Bleo	4NQO	
I-1	M/82	Fair/blue/red	9.0	0.15	1.06	0.42	1.87 ± 0.13
I-2	F/78	Fair/blue/blonde	52.0	0.76	1.12	0.24	2.90 ± 0.20
II-1	M/59	Fair/blue/blonde	35.0	0.57	0.93	0.73	1.53 ± 0.12
II-2	F/54	Olive/blue/dark brown	46.0	0.64	1.43	0.89	2.39 ± 0.17
II-3	F/58	Fair/blue/blonde	46.0	0.59	1.54	1.06	1.35 ± 0.12
II-4	ND[b]	–	–	–	–	–	–
II-5	F/58	Olive/blue/dark brown	20.0	0.26	1.34	0.43	1.86 ± 0.15
II-6	ND	–	–	–	–	–	–
II-7	M/52	Olive/blue/dark brown	10.0	0.10	1.00	0.34	2.16 ± 0.13
II-8	F/53	Fair/blue/blonde	3.0	0.07	0.33	0.27	2.27 ± 0.18
III-1	M/28	Olive/hazel/dark brown	36.0	0.34	1.45	0.68	1.48 ± 0.16
III-2	F/25	Olive/hazel/dark brown	13.0	0.13	0.82	0.75	1.37 ± 0.11
III-3	ND	–	–	–	–	–	–
III-4	ND	–	–	–	–	–	–
III-5	ND	–	–	–	–	–	–
III-6	ND	–	–	–	–	–	–
III-7	M/30	Fair/blue/blonde	15.0	0.13	0.48	0.40	ND
III-8	M/24	Fair/hazel/blonde	22.0	0.18	0.72	0.30	2.66 ± 0.19
III-9	M/22	Fair/blue/blonde	24.0	0.20	1.29	0.23	2.58 ± 0.16

[a] SE, standard error.
[b] ND, not done.

Pathak et. al., 2004

Werner Syndrome

- Rare autosomal recessive disorder (1 per million individuals)

- Loss of function mutations in Wrn gene (RecQ DNA helicase)
  DNA recombination, replication, maintenance of genome stability

15-years-old 48-years-old

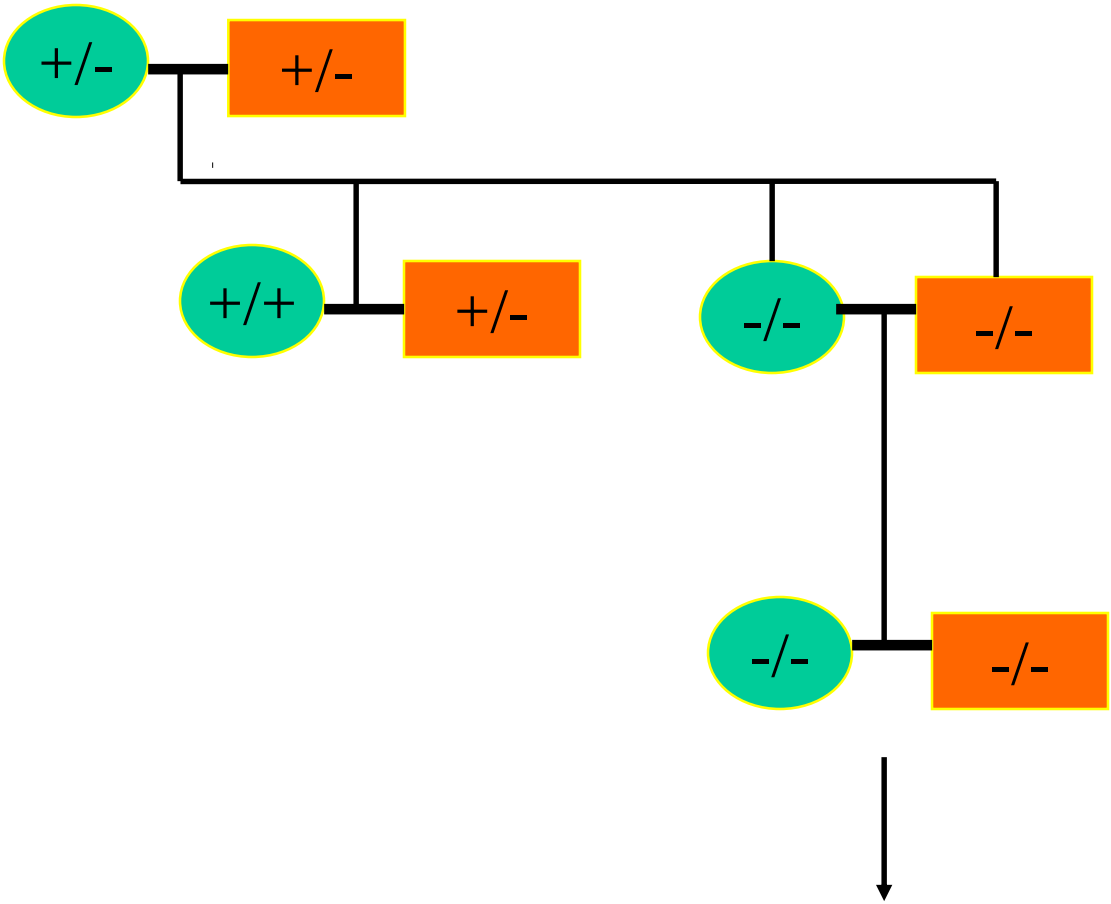
Age-related phenotypes:

- Cataracts
- Graying of hair
- Thinning of skin & ulceration
- Osteoporosis/fractures
- Hypogonadism
- Diabetes (type II)
- Atherosclerosis
- Cancer-prone:
  - Soft tissue sarcoma
  - Osteosarcomas
  - Thyroid CA

Average Lifespan 46-48 yrs

- Understanding this model of pathological aging may shed light on the molecular basis of normal human aging.

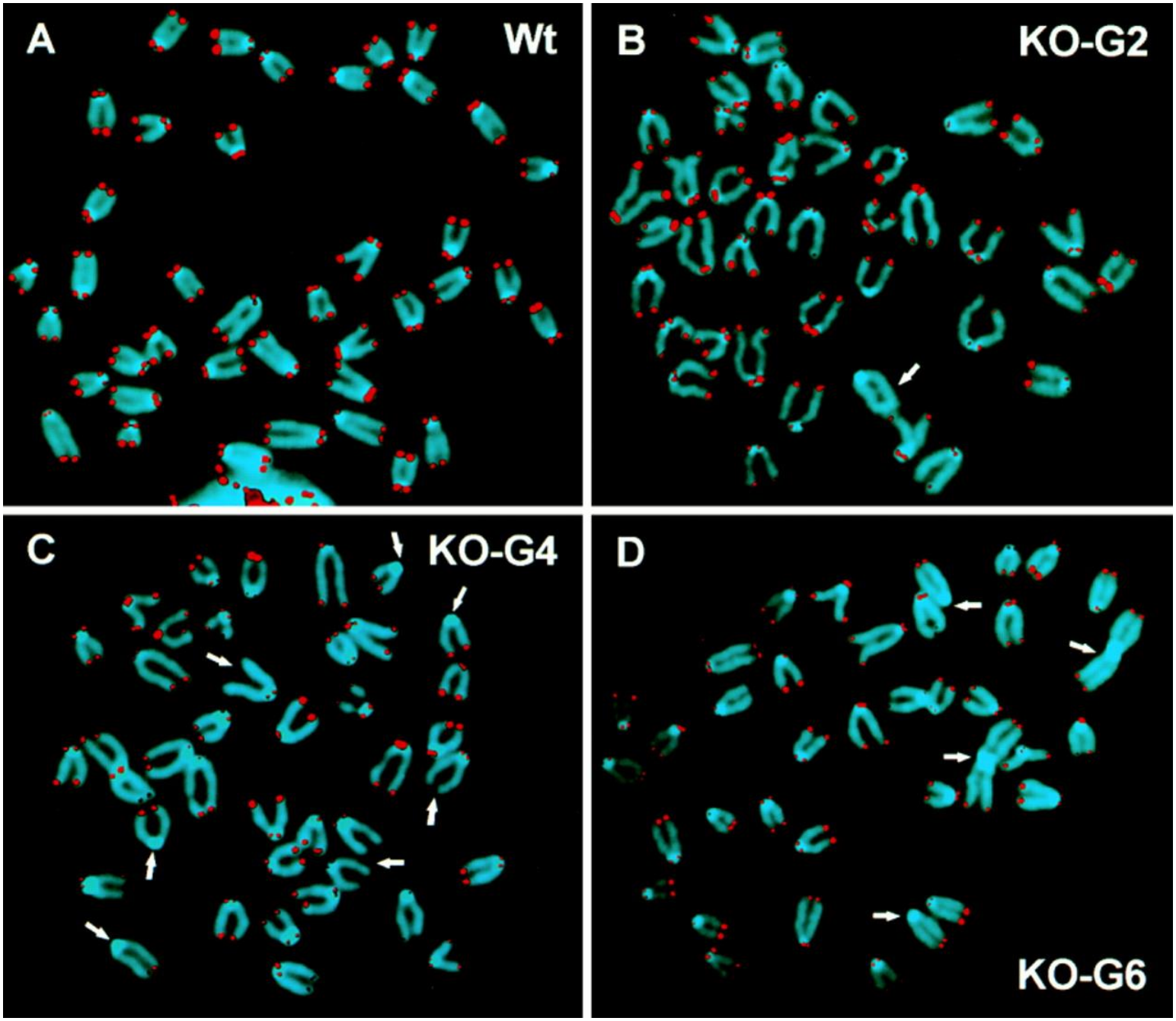
Mating scheme to generate mTERC-/- mice



G1 long telomeres

G2 shorter telomeres

G6 or G7 critically short telomeres

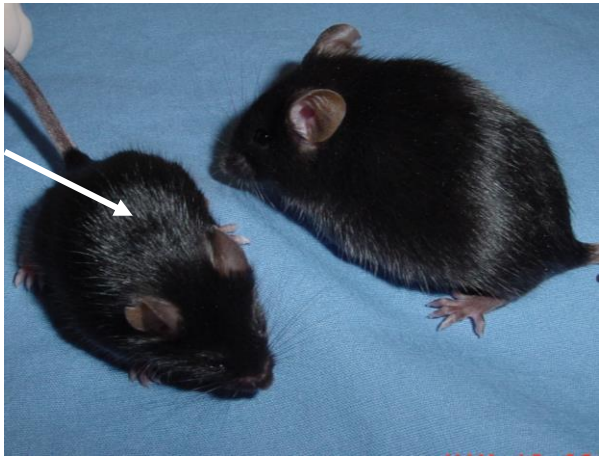

A
Wt
B
KO-G2
C
KO-G4
D
KO-G6

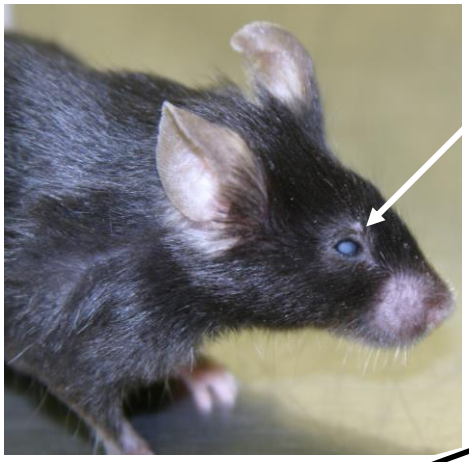
Clinical Presentation of mTERC-/-Wrn-/- Mutant Mice

G1-3 mTERC-/- WRN+/+ or -/-
G4-6 mTERC-/- WRN+/+ } No WS Phenotype

G4-6 mTERC-/- WRN-/- (70% penetrant by 8 months of age)

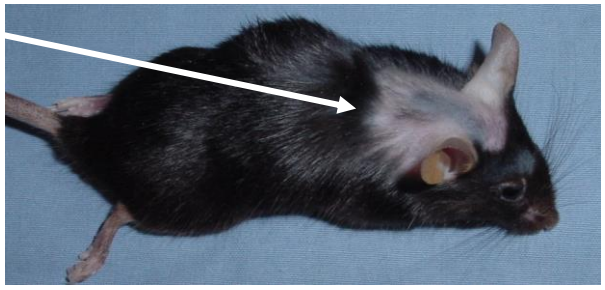
stunted growth

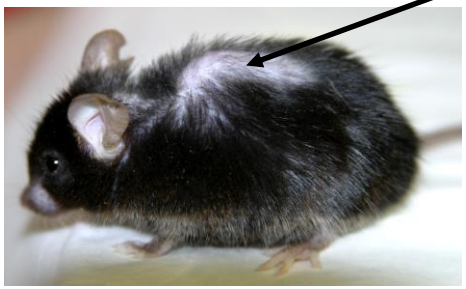




cataract

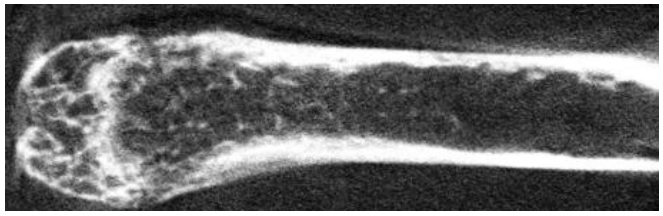
alopecia & hair greying



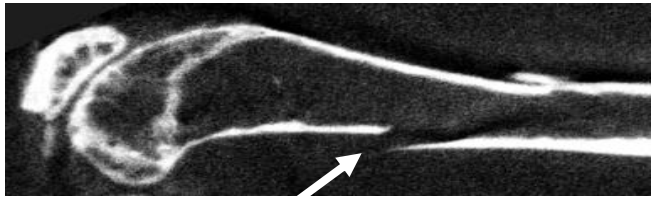


kyphosis

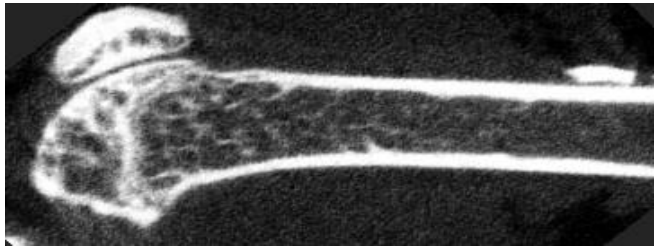
Osteoporosis & Pathological Fractures in Adult G4-6 mTERC-/- WRN-/- Mice



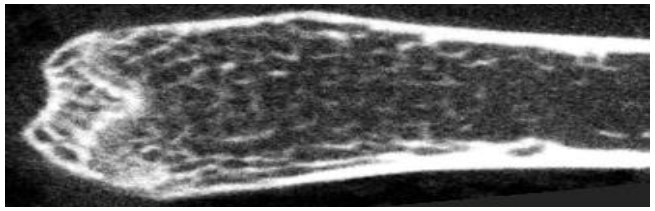
G5 Wrn+/+ (8 months)



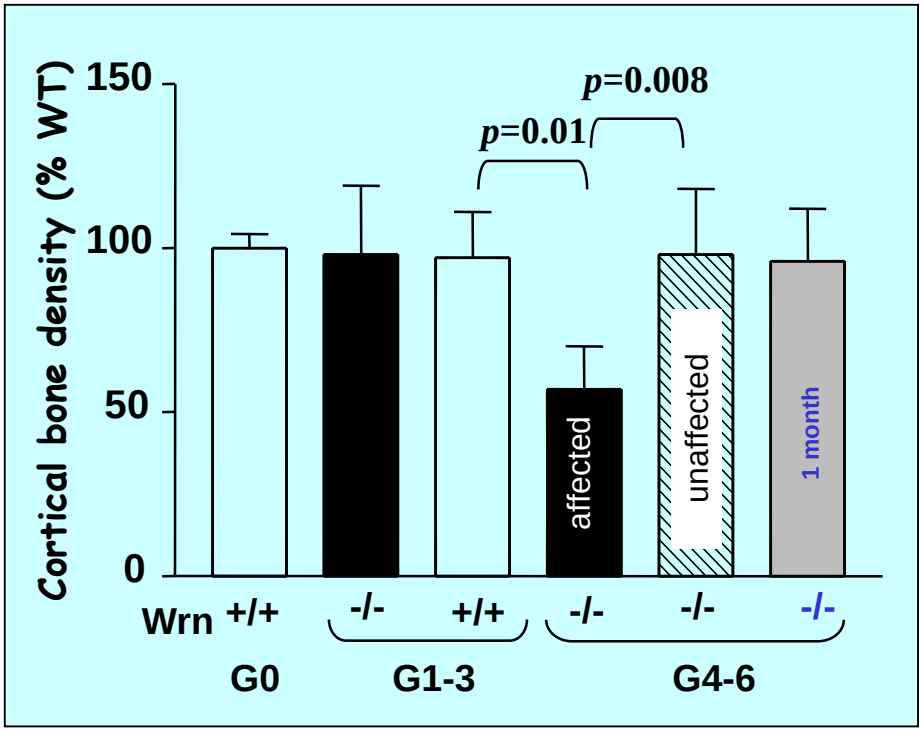
G5 Wrn-/- affected (8 months)



G5 Wrn-/- unaffected (8 months)



G5 Wrn-/- (1 month)



Kaplan-Meier Analysis of Overall Survival

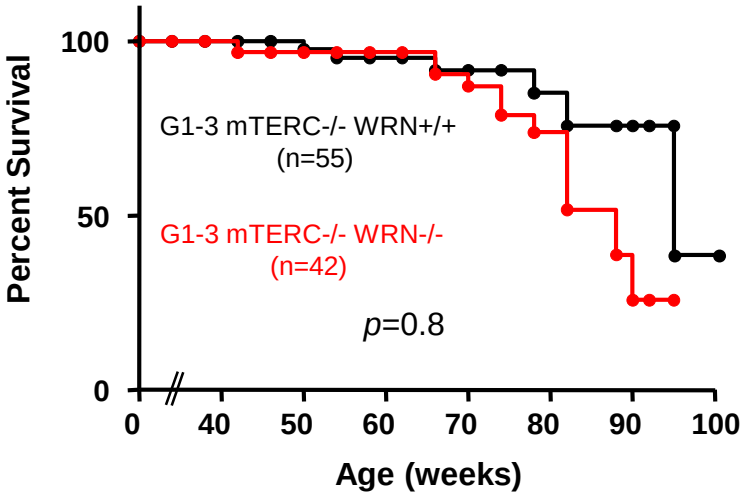
Hypothesis: manifestation of WS phenotypes in mice requires critical telomere shortening.

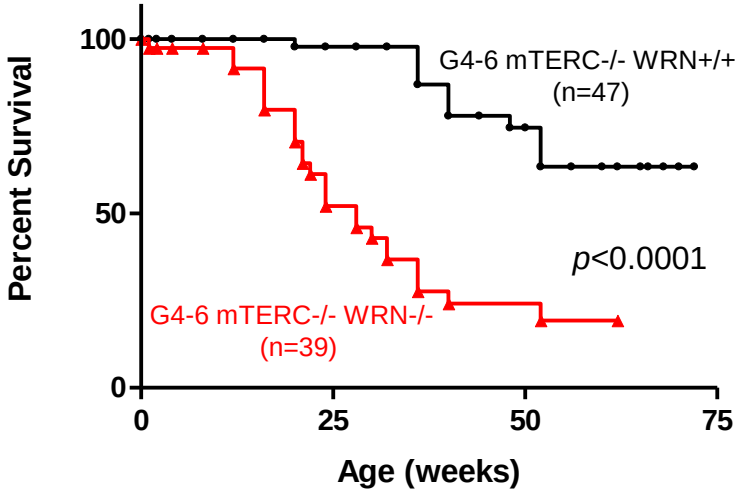
1. G1-G3 mTERC-/- Wrn+/+
2. G1-G3 mTERC-/- Wrn-/-

} long telomeres

3. G4-G6 mTERC-/- Wrn+/+
4. G4-G6 mTERC-/- Wrn-/-

} short, dysfunctional telomeres





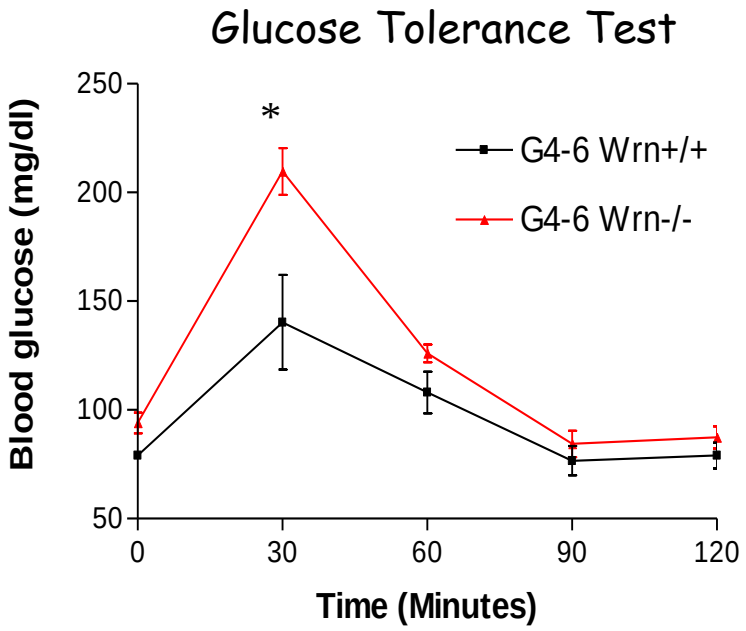
G1-3 mTERC-/- Wrn+/+
G1-3 mTERC-/- Wrn-/-

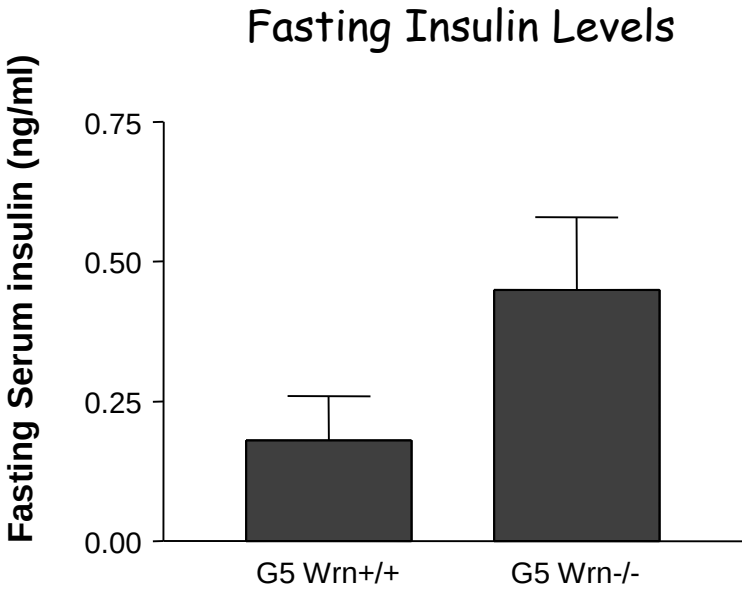
} ~90+/- 4 wks

G4-6 mTERC-/-Wrn+/+ : 81 +/- 9.2 wks

G4-6 mTERC-/-Wrn-/- : 24 +/- 5.6 wks

Glucose Homeostasis in mTerc & Wrn Mutant Mice

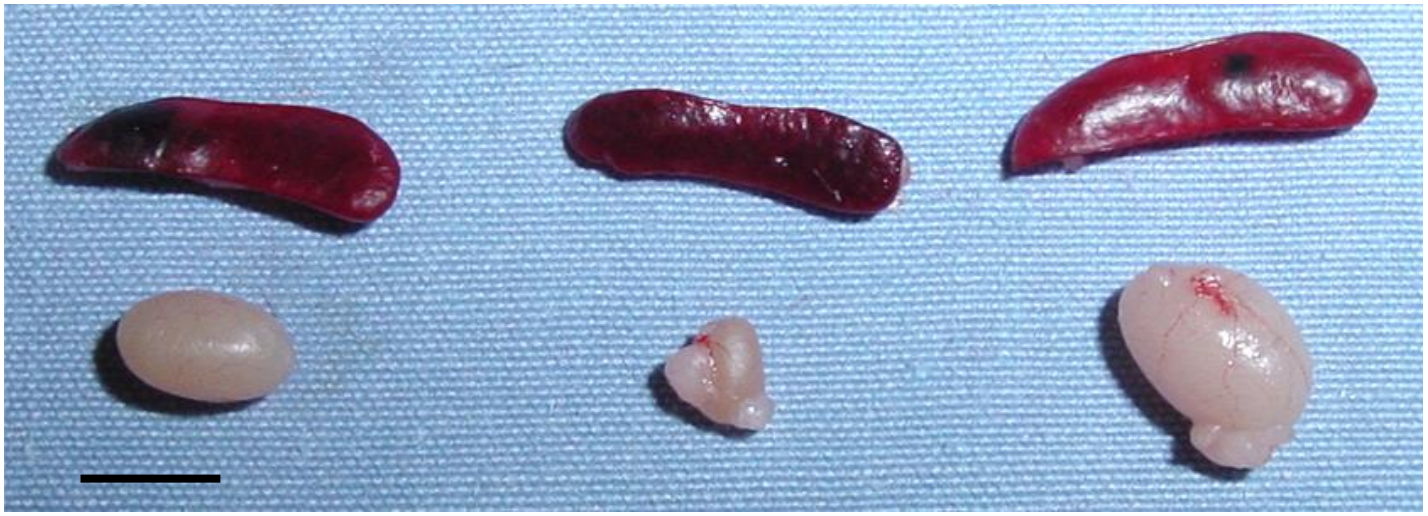




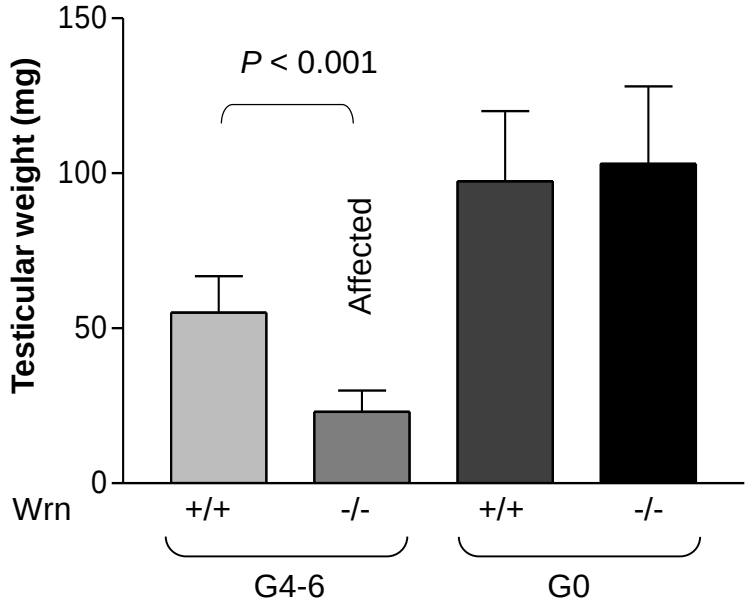
Elevated Fasting Glucose
Abnormal Glucose Tolerance
Elevated Fasting Insulin

→ Type II Diabetes

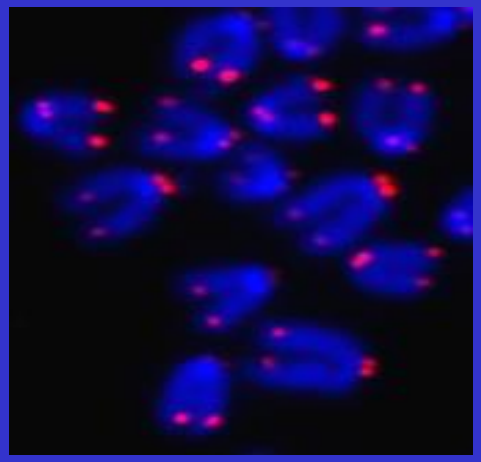
Hypogonadism in G4-6 mTerc-/- Wrn-/- mice



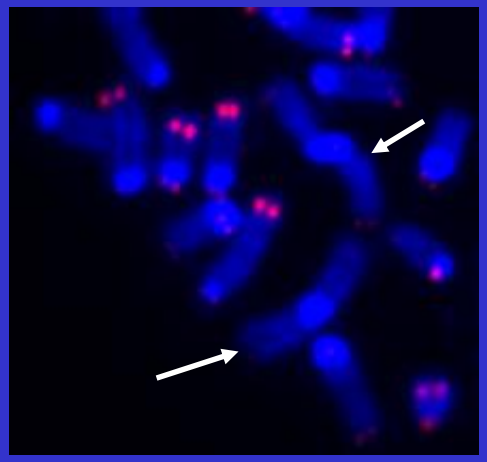
G4 mTERC$^{-/-}$ Wrn$^{+/+}$ G4 mTERC$^{-/-}$ Wrn$^{-/-}$ G0 mTERC$^{+/-}$ Wrn$^{-/-}$



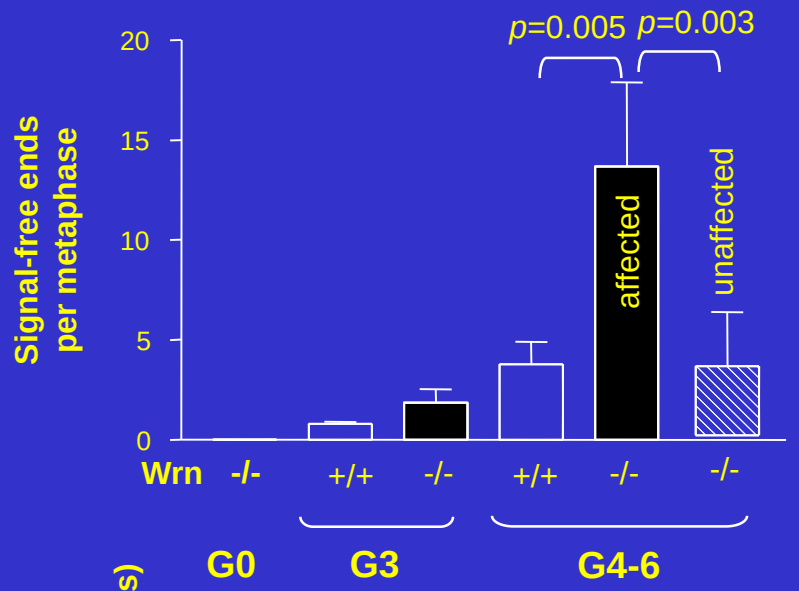
Increased Signal-Free Ends and Decreased Telomere Length in G4-6 mTERC-/- WRN-/- Bone Marrows

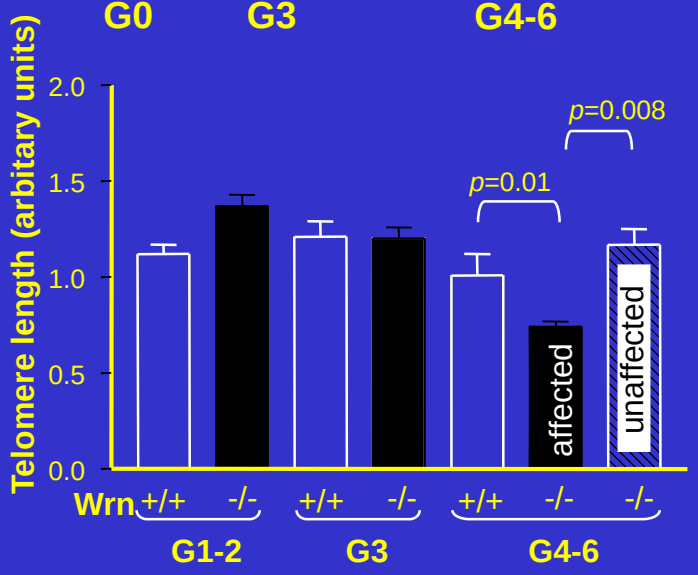


G5 Wrn+/+

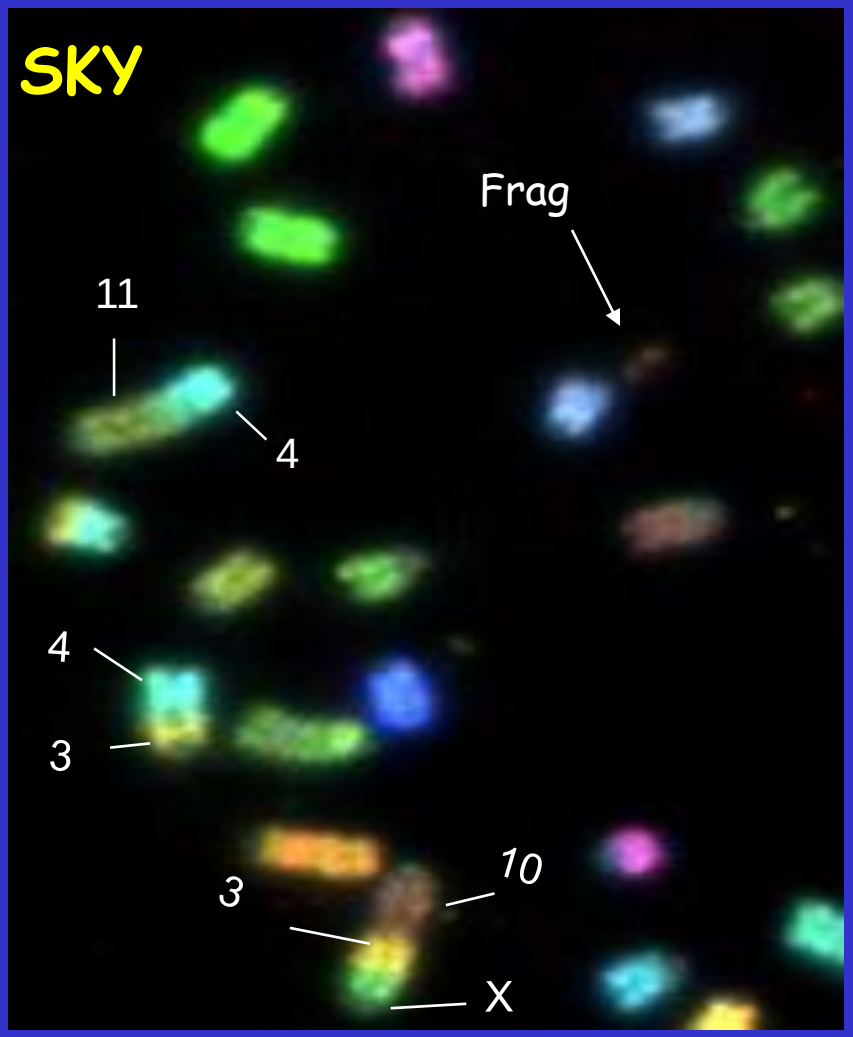


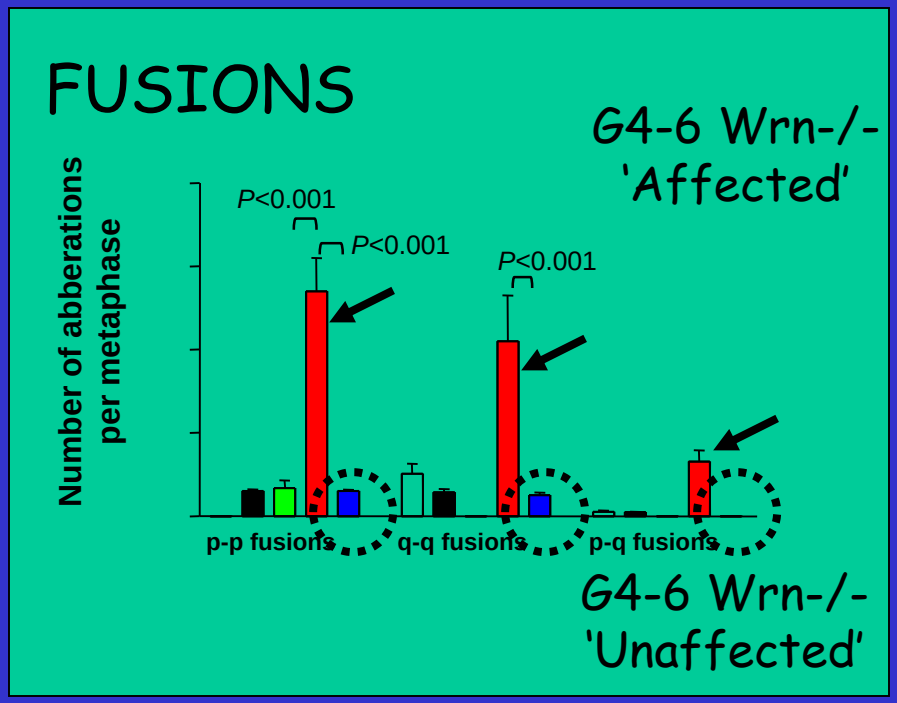
G5 Wrn-/-



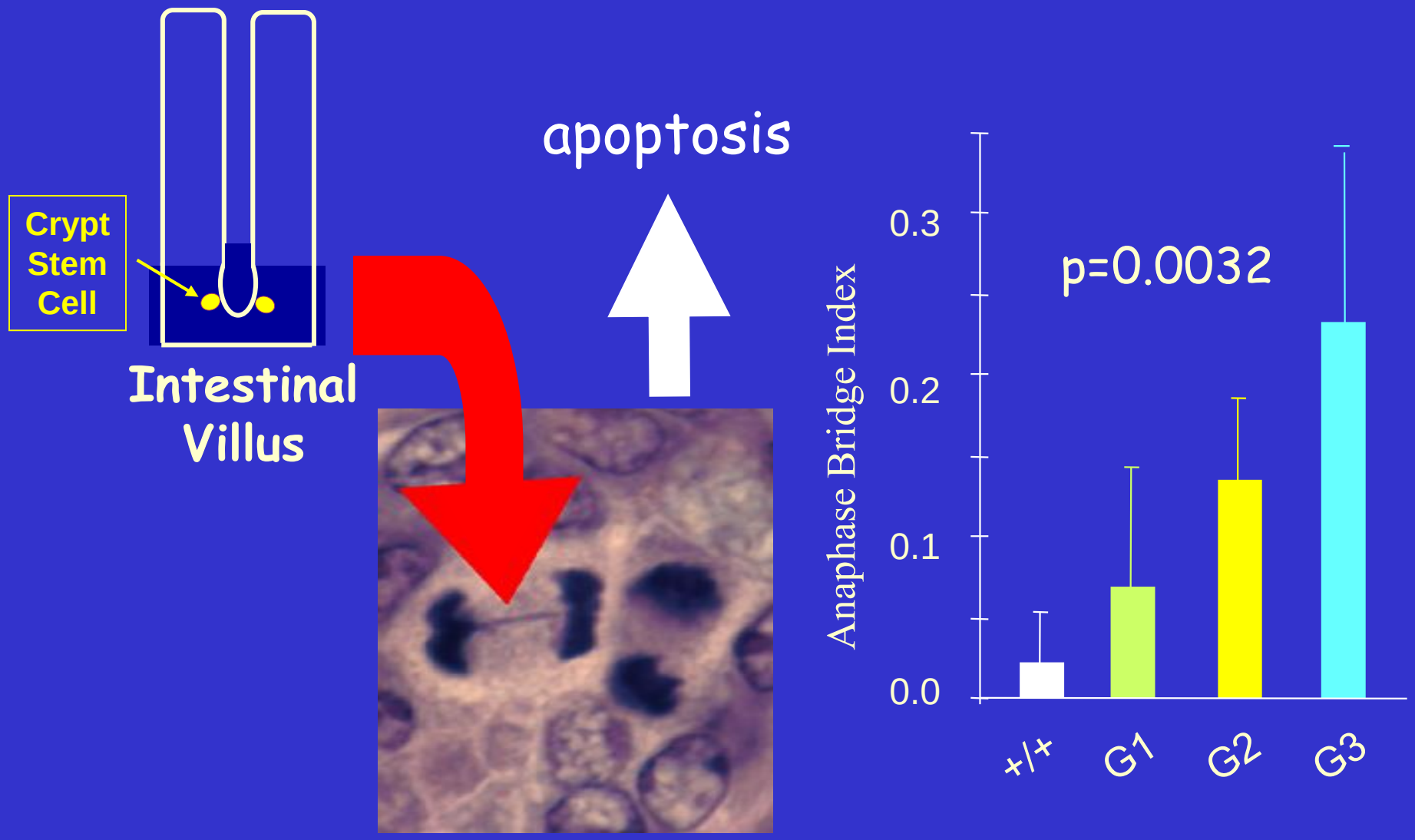


Chromosomal Fusions & Structural Aberrations in G4-6 mTERC-/- WRN-/- Mice

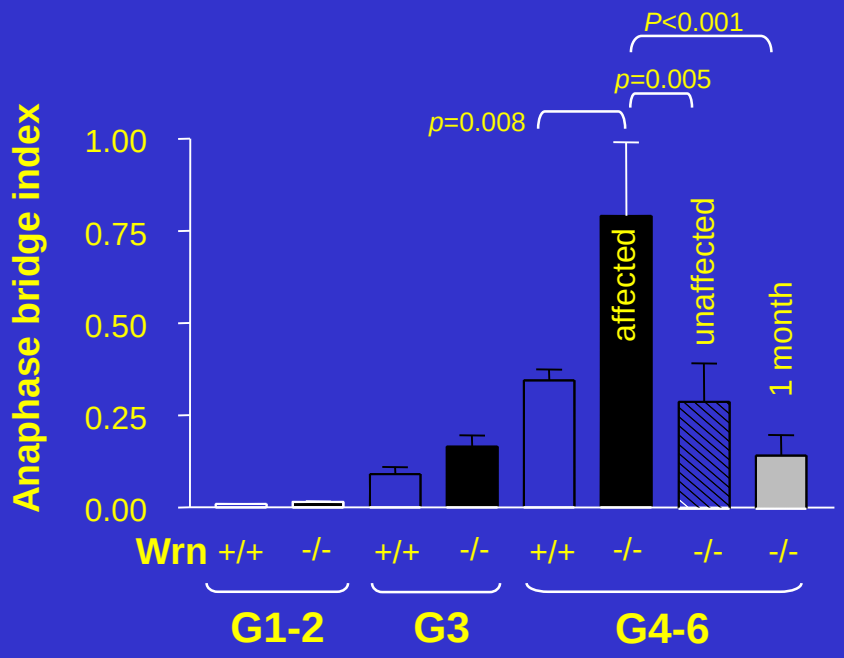


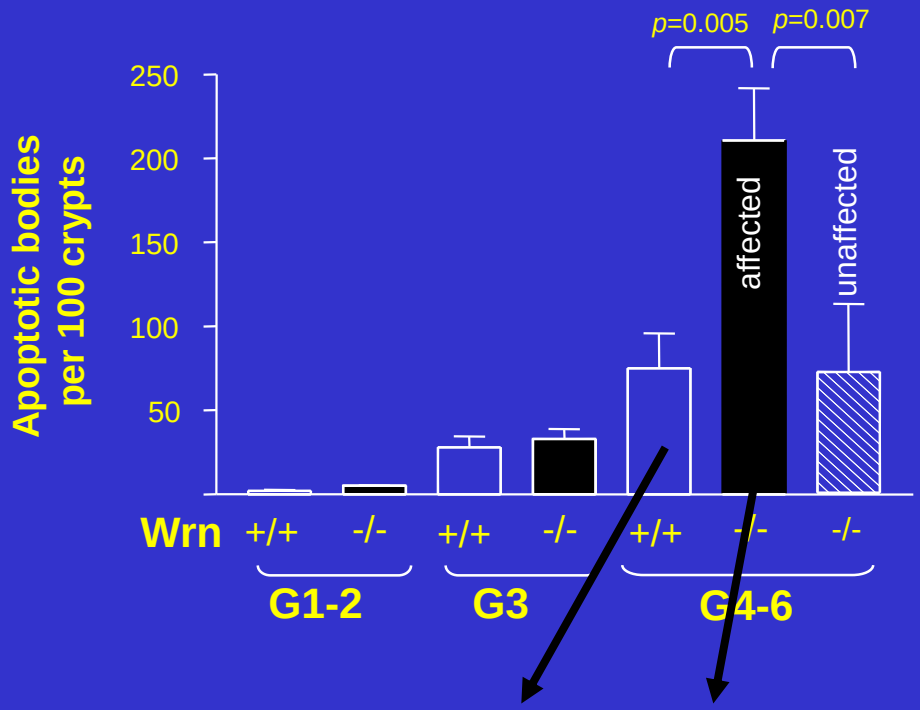


Anaphase bridge index correlates with the level of telomere dysfunction

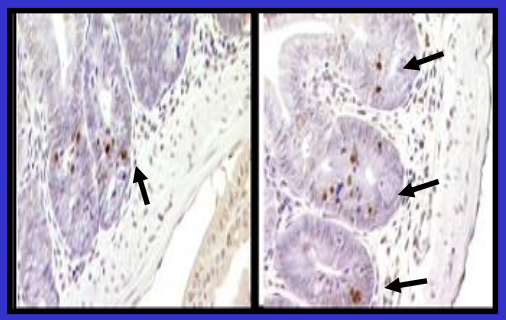


Clinical Aging Correlates With Intestinal Crypt Apoptosis and Anaphase Bridging



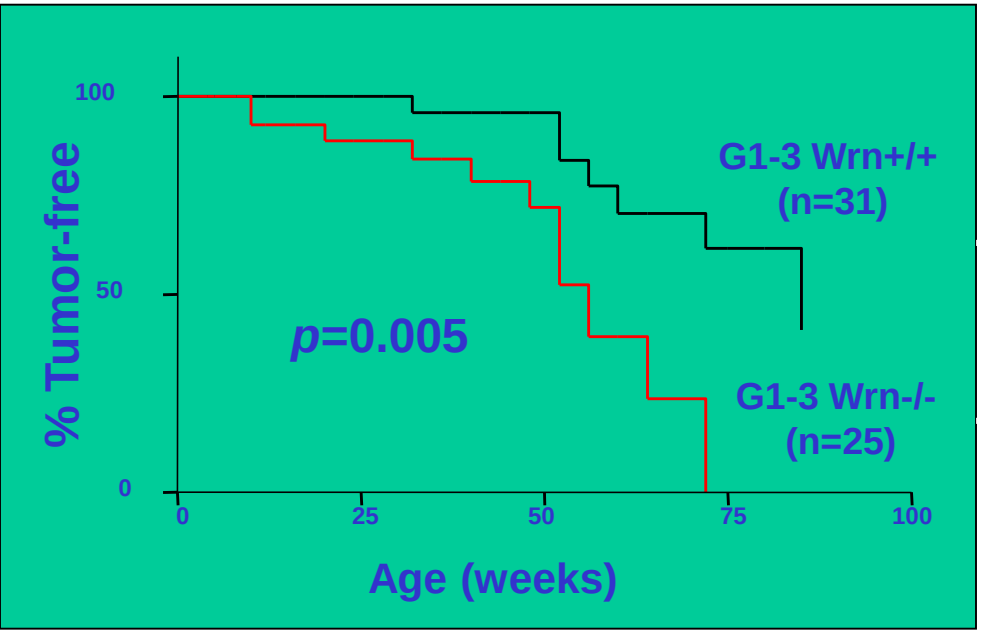


TUNEL assay



G5 Wrn+/+ G5 Wrn-/-

Neoplastic Impact of WRN & Dysfunctional Telomeres



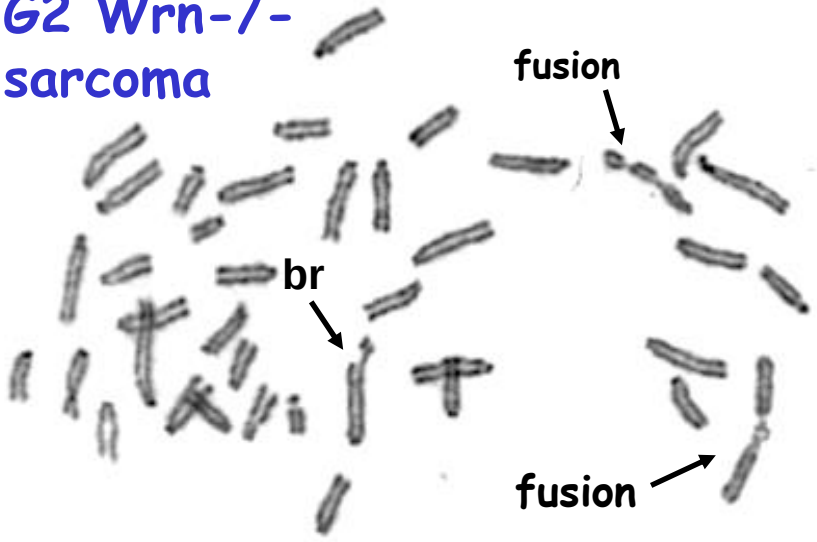
G1-3 mTerc-/-Wrn+/+

Lymphoma	**68%**
Soft tissue sarcoma	**16%**
Osteosarcomas	13%
Other tumors	3%

G1-3 mTerc-/-Wrn-/-

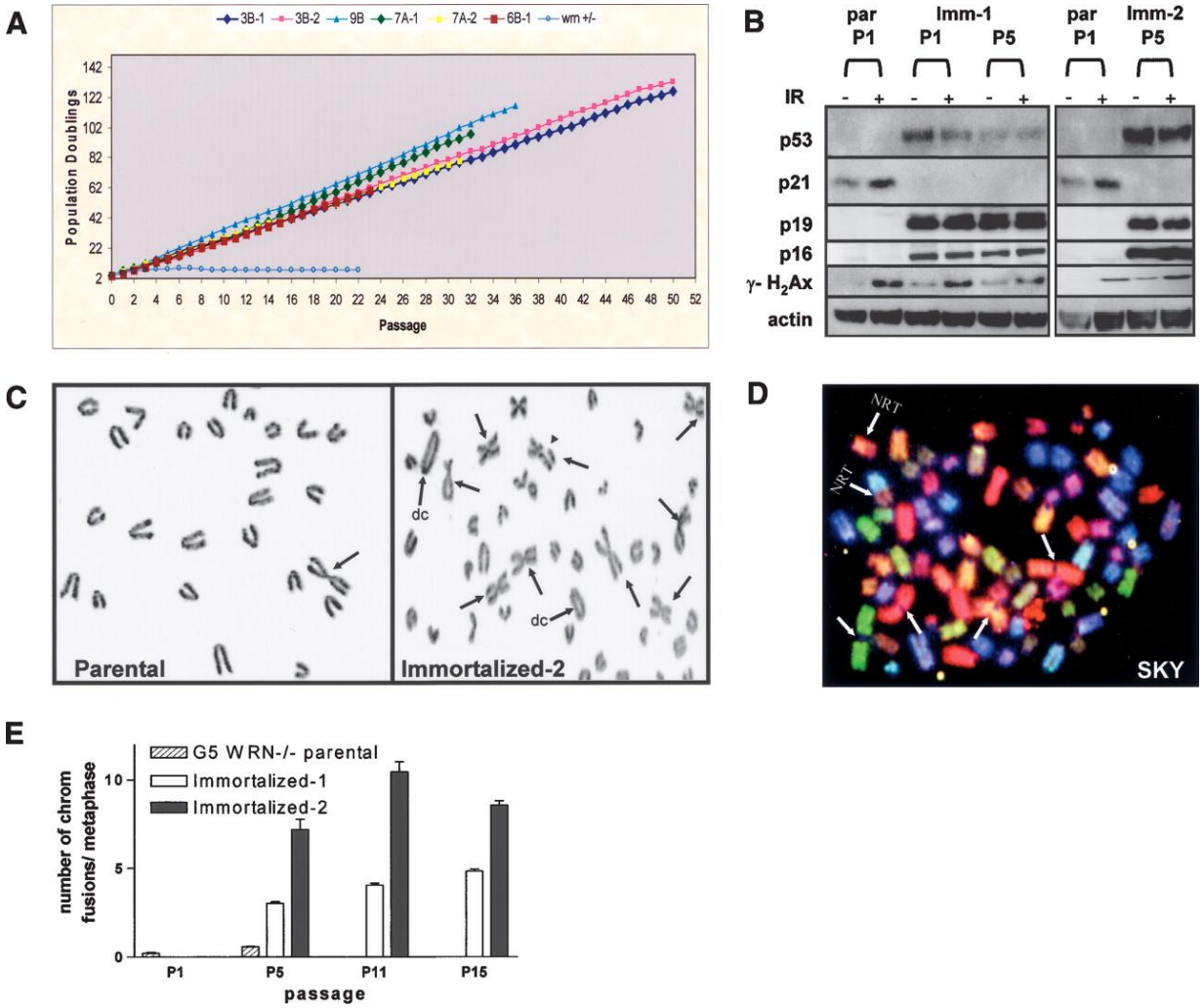
Osteosarcoma	**52%**
Soft tissue sarcoma	**24%**
Lymphoma	16%
Carcinomas	8%

G2 Wrn-/- sarcoma



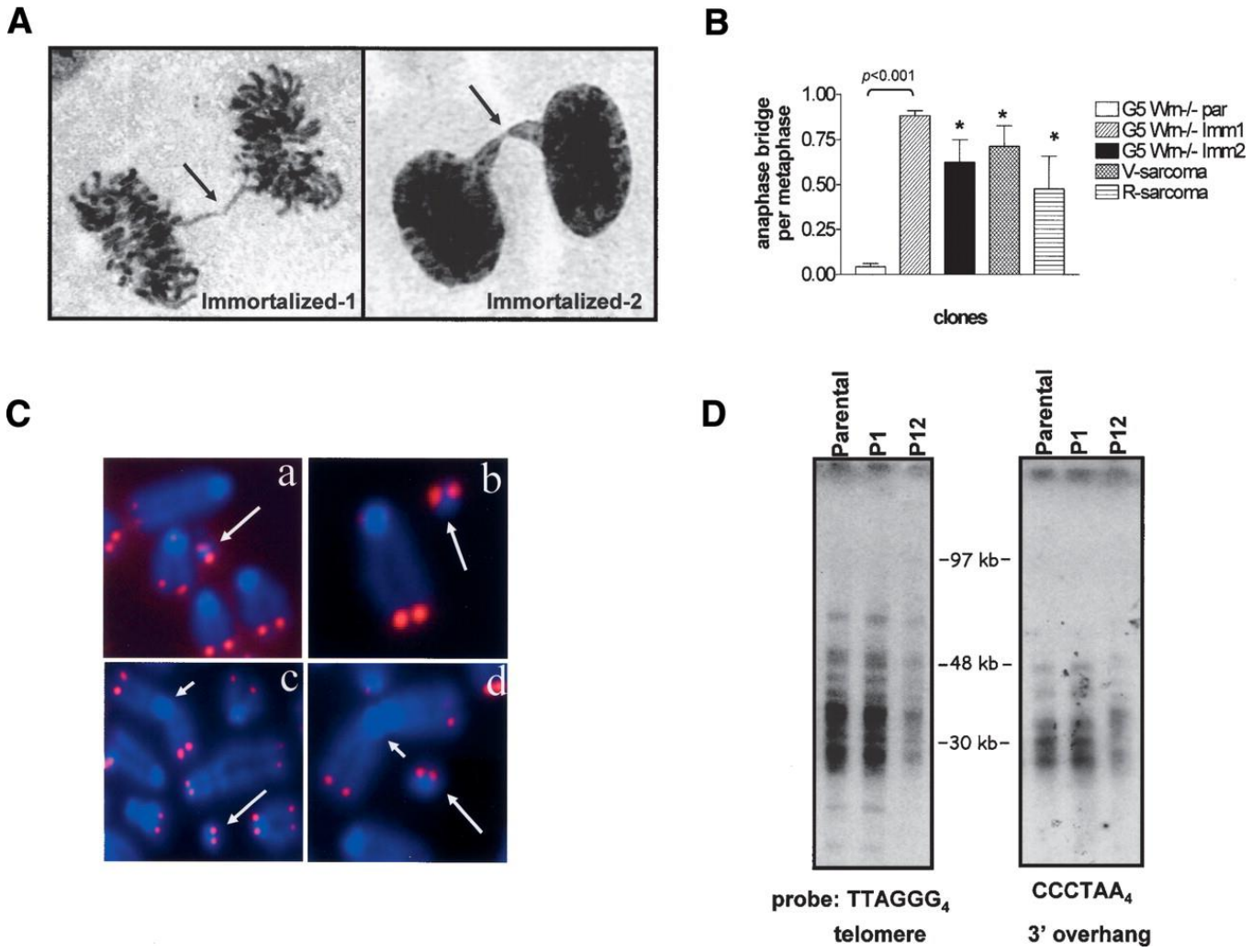
- Increased Cancer Incidence
  85 → 62 weeks
- Shift towards WS Spectrum
  Lymph → Osteosarcomas
- Chromosomal Aberrations
  Fusions, Breaks & NRTs

Figure 1. G5 mTerc-/- Wrn-/- MEFs spontaneously immortalize and exhibit chromosomal aberrations



Purnima R. Laud et al. Genes Dev. 2005; 19: 2560-2570

Figure 2. Characterization of telomere structure and TDMs in G5 mTerc-/- Wrn-/- immortalized clones



Purnima R. Laud et al. Genes Dev. 2005; 19: 2560-2570

Tumorigenic potential of immortalized MEF's

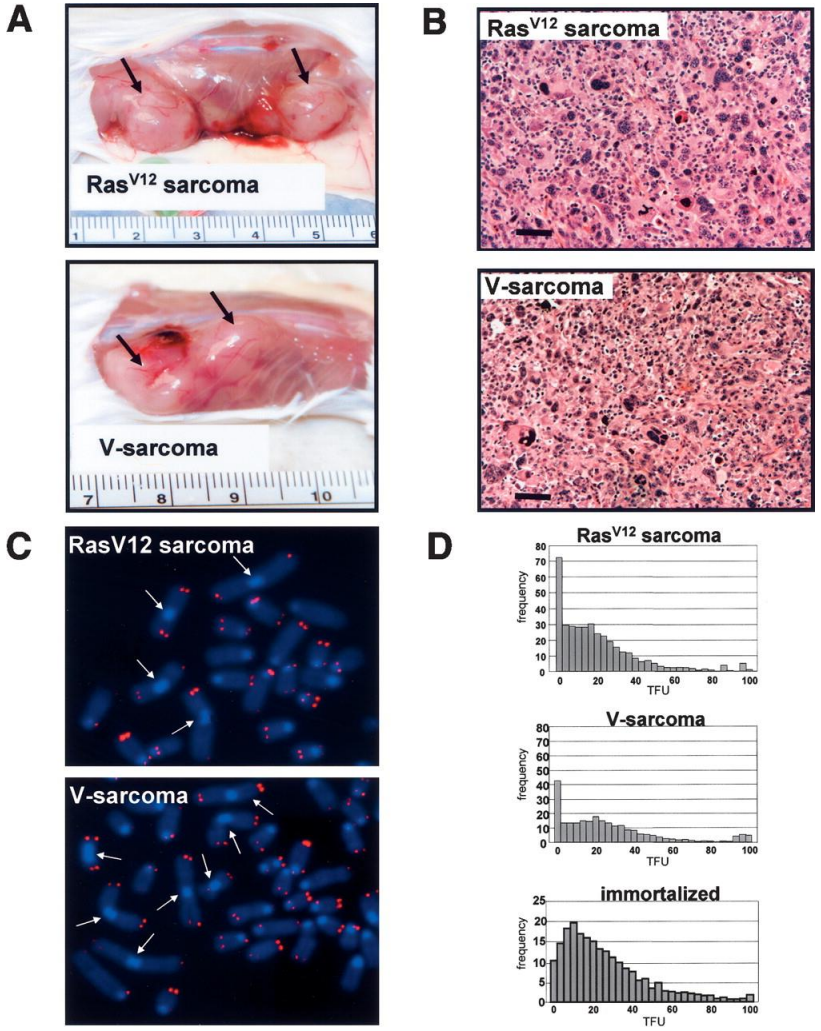
- Immortalized MEF's were transfected with H- ras and injected in SCID mice.

Summary of SCID mice S.C. tumor injection

Cell lines

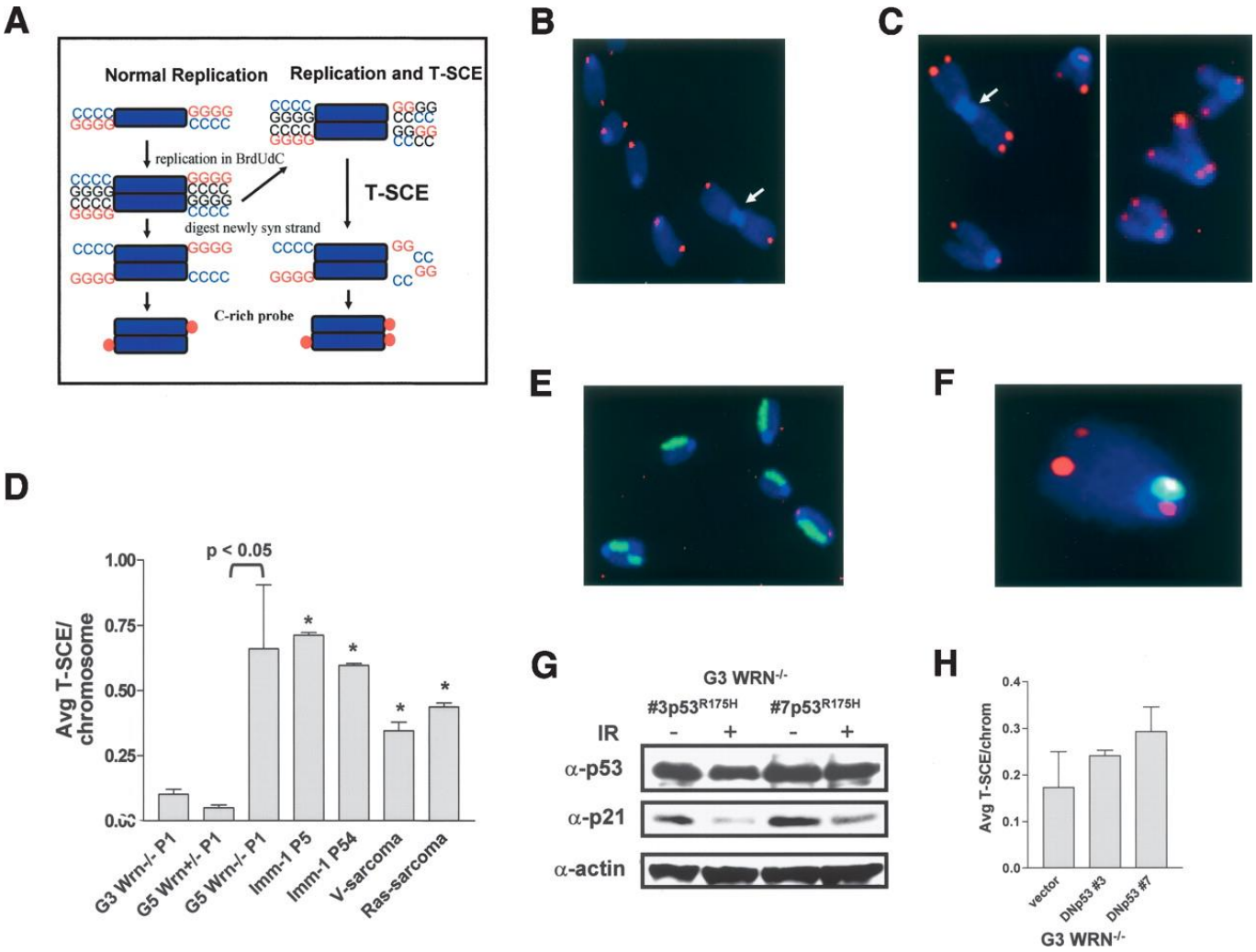
	sites	tumors	days	
Parental G5 m/Terc$^{-/-}$Wrn$^{-/-}$#1 P1	4	0	n.a.	
Parental G5 m/Terc$^{-/-}$Wrn$^{-/-}$#2 P2	8	0		
Parental G5 m/Terc$^{-/-}$Wrn$^{-/-}$#3 P1	4	0		
Immortalized G5 m/Terc$^{-/-}$Wrn$^{-/-}$ + vector #1 P2	8	8	43 d	
Immortalized G5 m/Terc$^{-/-}$Wrn$^{-/-}$ + vector #2 P2	8	6	42 d	
Immortalized G5 m/Terc$^{-/-}$Wrn$^{-/-}$ + vector #3 P3	8	6	48 d	
Immortalized G5 m/Terc$^{-/-}$Wrn$^{-/-}$ + vector #4 P5	8	4	41 d	
				$P < 0.004$
Immortalized G5 m/Terc$^{-/-}$Wrn$^{-/-}$ + H-Ras #1 P2	4	4	25 d	
Immortalized G5 m/Terc$^{-/-}$Wrn$^{-/-}$ + H-Ras #2 P2	4	2	26 d	
Immortalized G5 m/Terc$^{-/-}$Wrn$^{-/-}$ + H-Ras #3 P3	4	4	20 d	
				$P < 0.01$
Immortalized G5 m/Terc$^{-/-}$Wrn^{++} p53$^{-/-}$ #1 P3	4	0	n.a.	

Figure 5. Transformation and tumorigenic potential of immortalized G5 mTerc-/- Wrn-/- MEFs



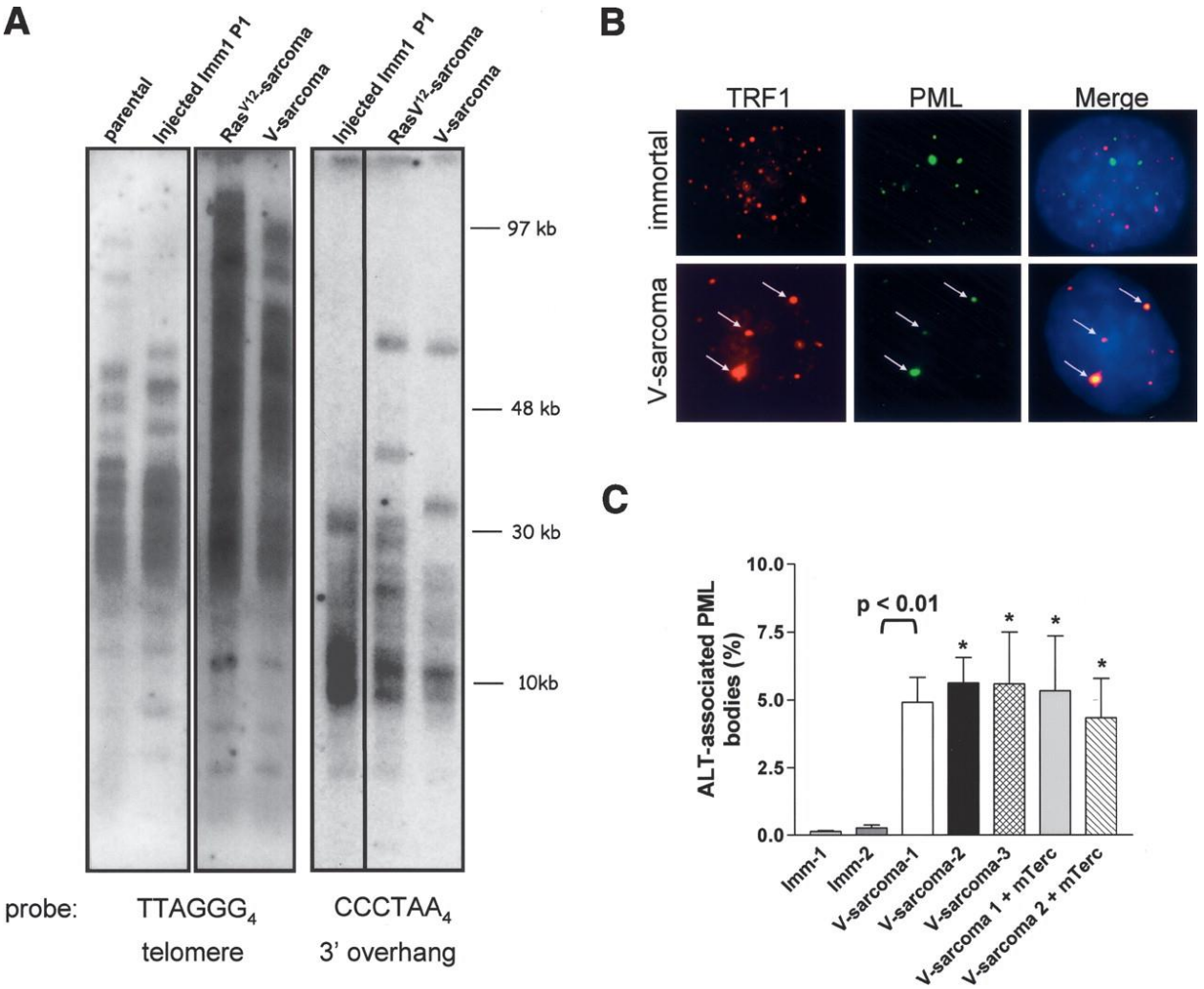
Purnima R. Laud et al. Genes Dev. 2005; 19: 2560-2570

Figure 3. T-SCE is elevated in G5 mTerc-/- Wrn-/- cells



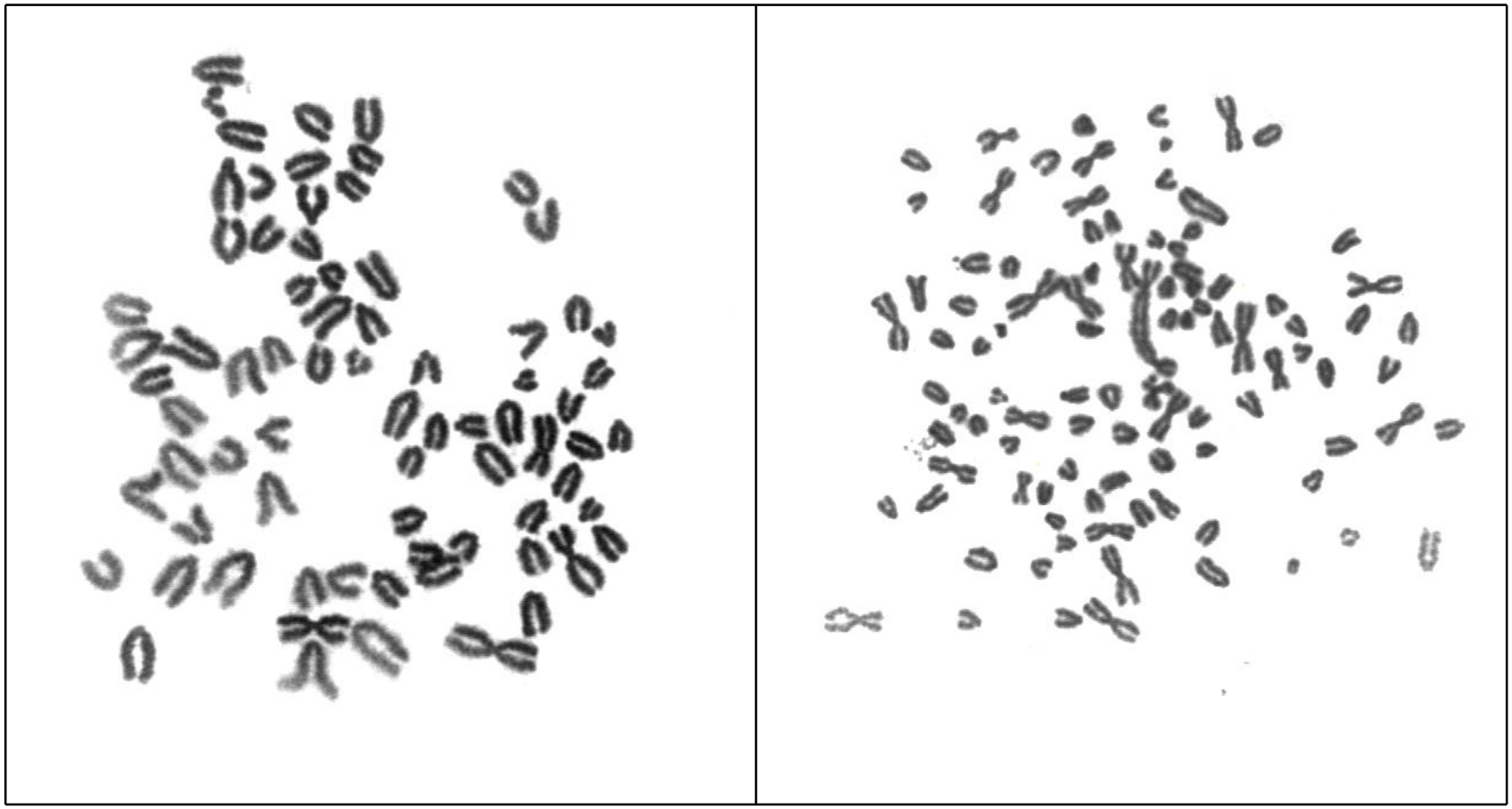
Purnima R. Laud et al. Genes Dev. 2005; 19: 2560-2570

Figure 6. Engagement of the ALT pathway in H-RasV12 and V-sarcomas

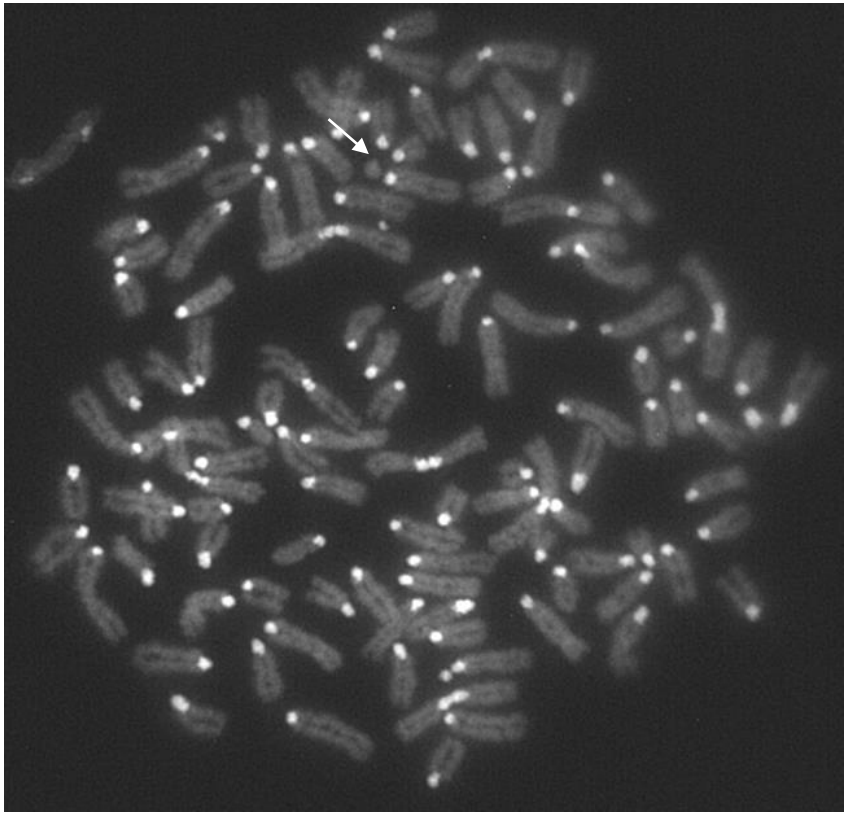


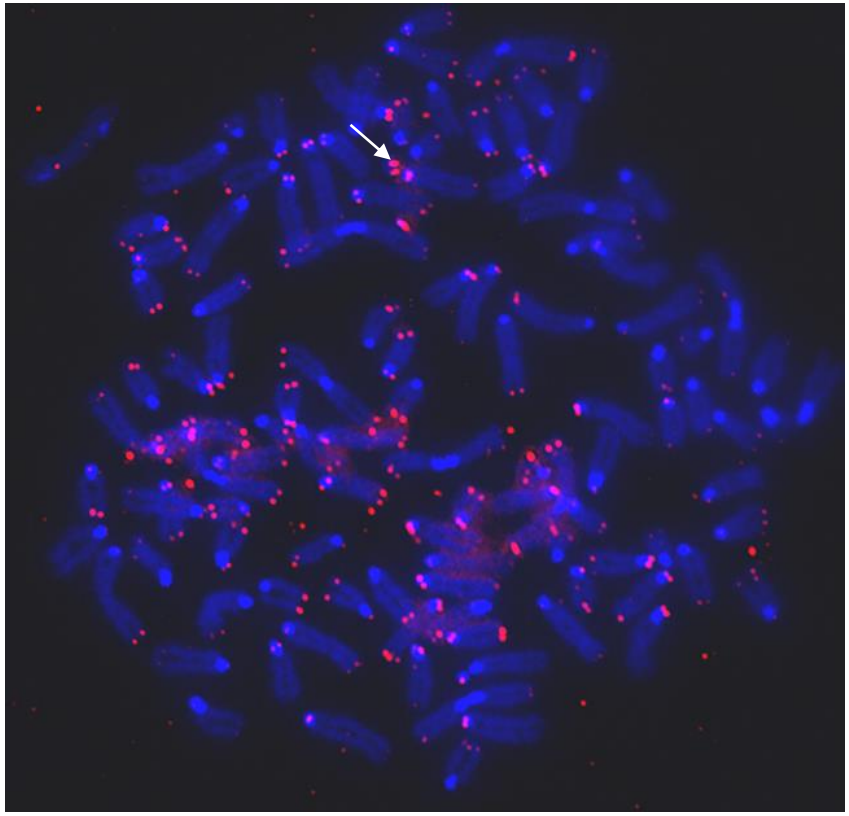
Purnima R. Laud et al. Genes Dev. 2005; 19: 2560-2570

Immortalized 9B-1 before and after injection in SCID mice

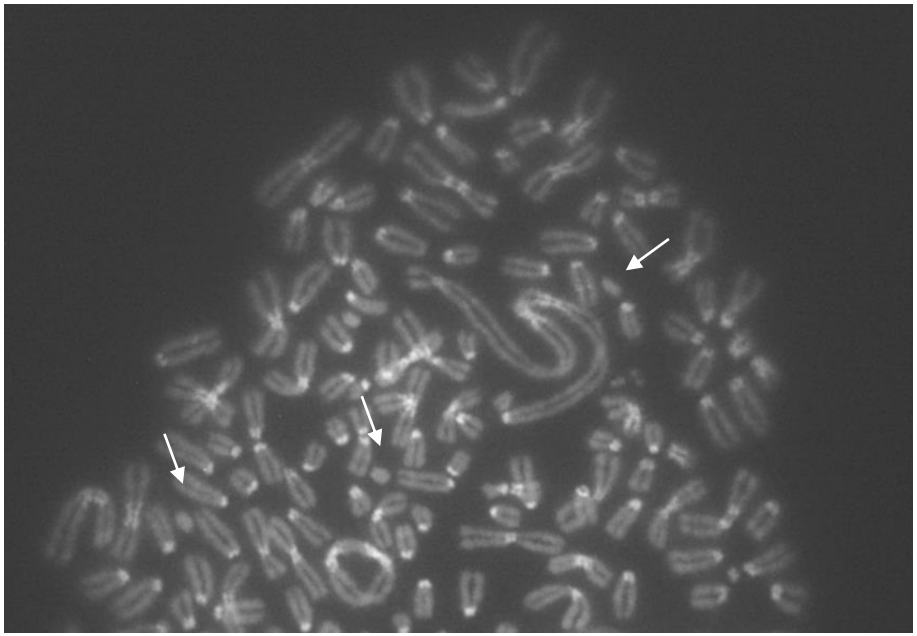


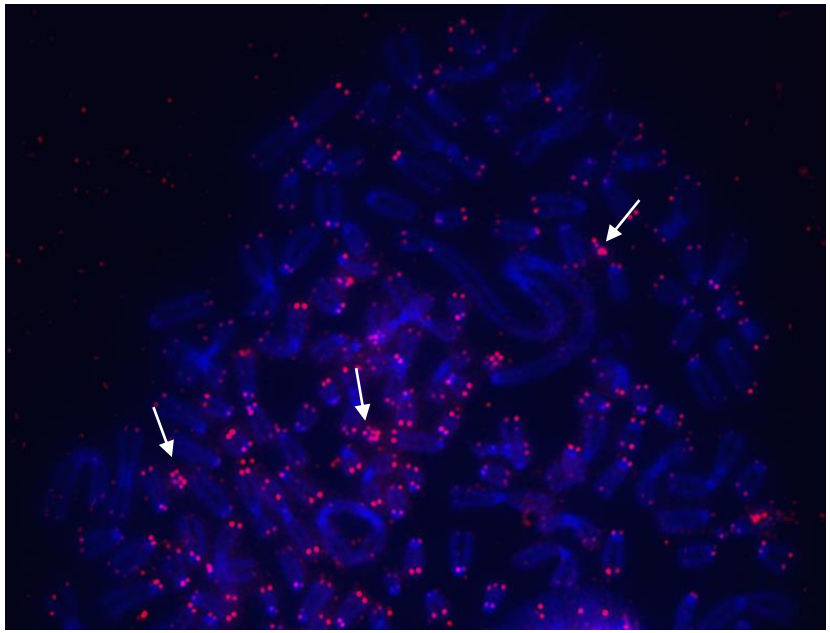
Immortalized MEF cells (9B-1)

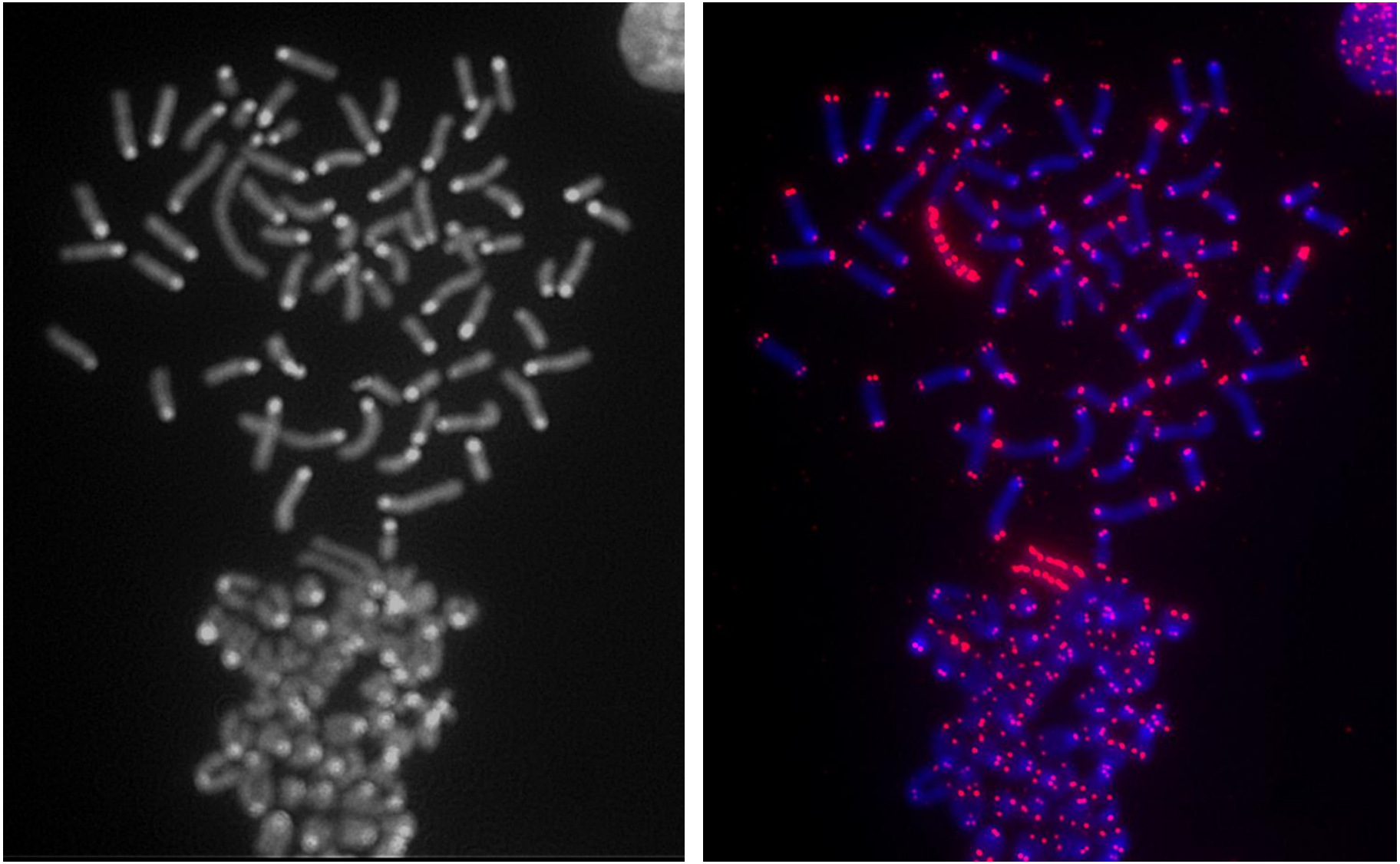




Tumor following 9B-1 injection in SCID mice







Tumor from a Pot1-a deleted mouse breast using MMTV-Cre

CONCLUSION

ShortTelomeres are required for WS Pathogenesis

Human WS	Wrn-/-	G4-6 Wrn+/+	G4-6 Wrn-/-
Osteoperosis	No	No	++++
Cataracts	No	No	++++
Type II Diabetes	No	No	++++
Skin defects	No	++	++++
Hypogonadism	No	++	++++
Atherosclerosis	No	No	No
Genome Instability	No	++	++++
Mesenchymal tumors	No	+	++++

CORD BLOOD TRANSFUSION
(vs Bone Marrow)

- Rich source of stem cells
- Cord blood can be pooled from different individuals and then transfused
- No matching is needed
- Survive longer than bone marrow cells
- Have much longer telomere repeats

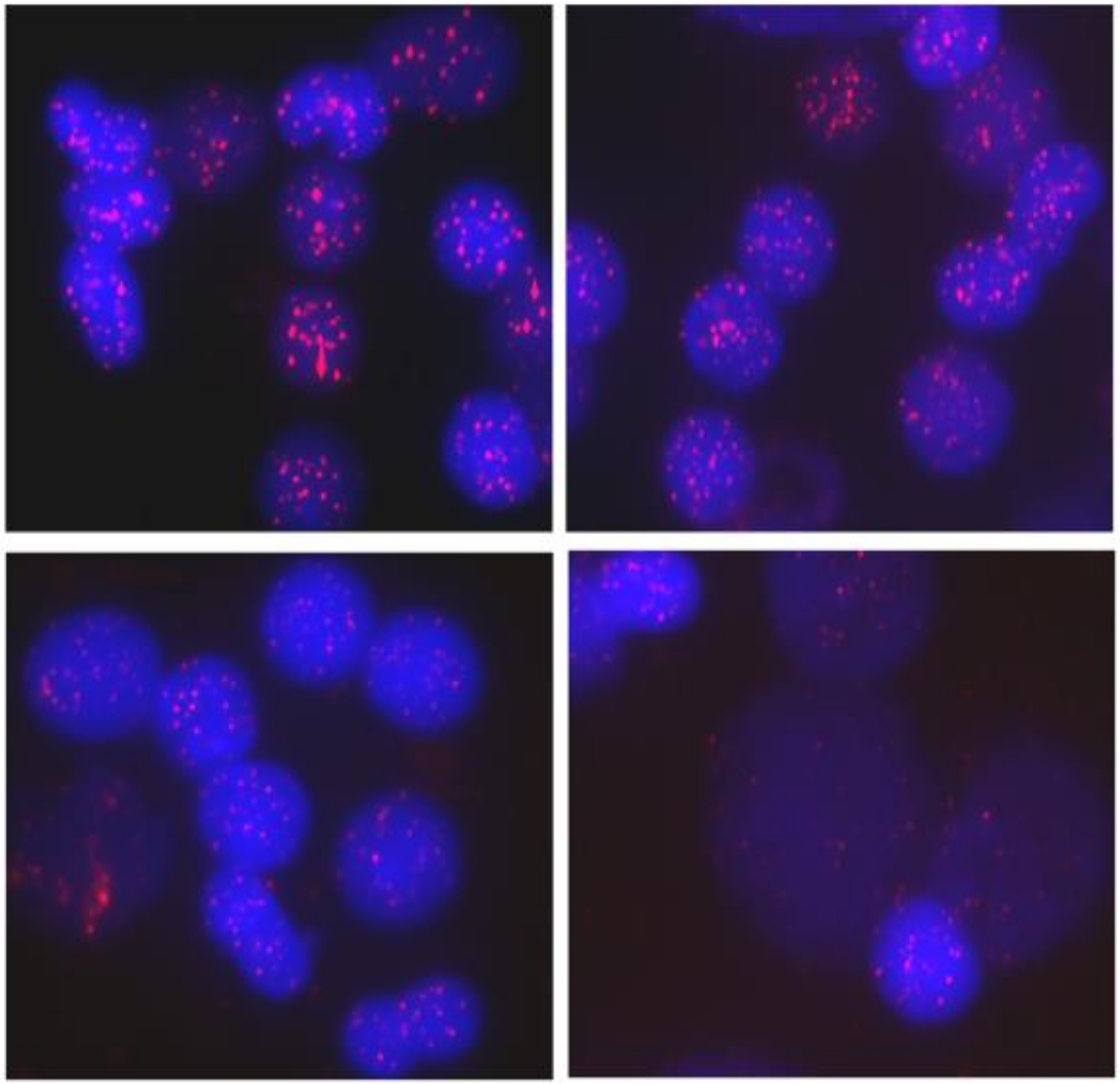
Table I. Q-FISH studies in Bone marrow transplant cases

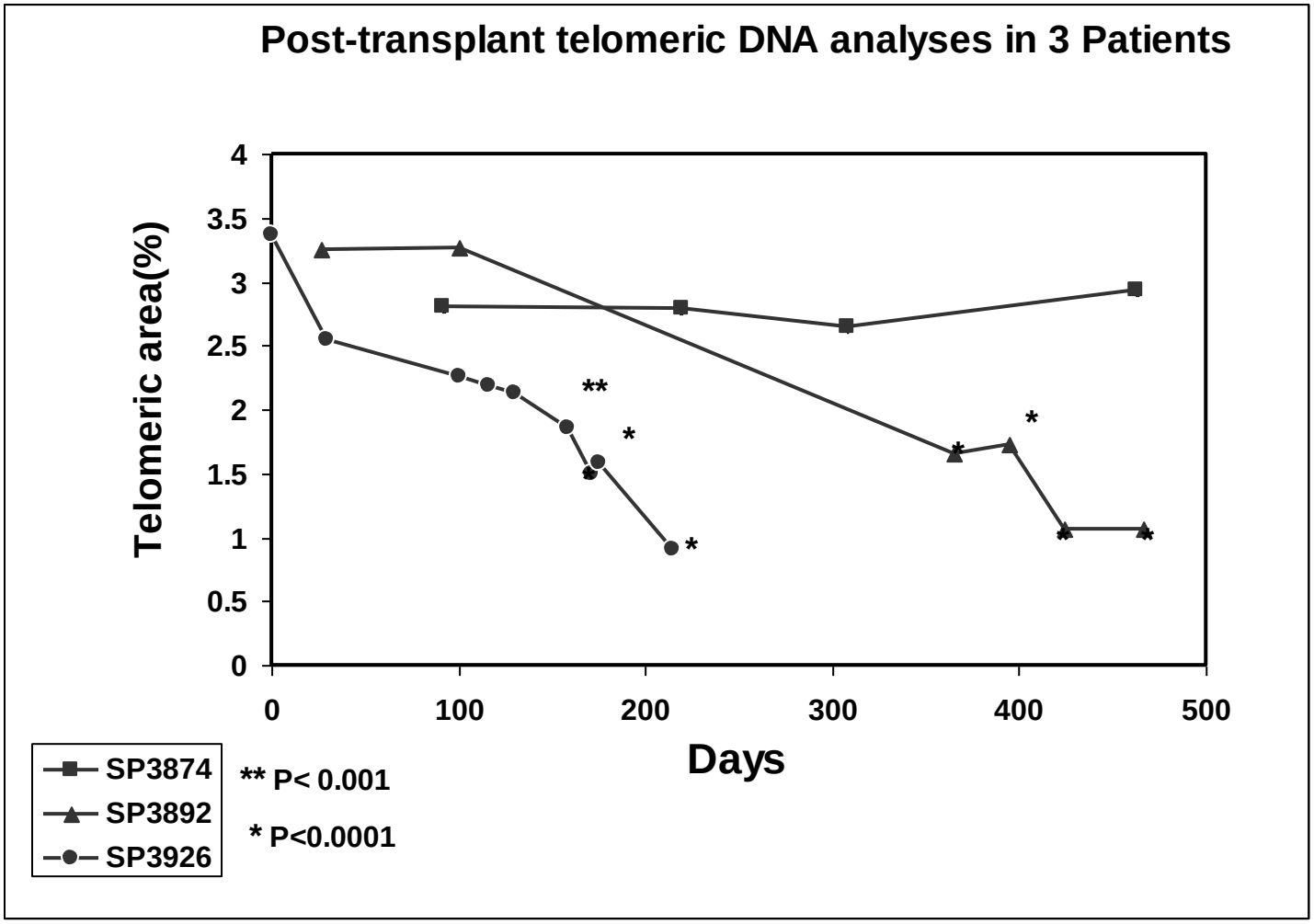
	DATE REC'D	SAMPLE #	MDACC #	DOB	SAMPLE TYPE	% Telomeric Area	
1	12/7/99	SP 3772 I	378128	8/31/94	BM 100 Days post	1.61	deceased
2	12/9/99	SP 3773 I	406538	10/11/91	BM 31 Days post	1.62	deceased
	2/16/00	SP 3773 II			BM 103 Days post	1.3	
3	12/10/99	SP 3774 I	354726	9/25/89	blood		
	5/15/00	SP 3774 II			pretransplant	2.51	
	1/25/01	SP 3774 III			BM 103 Days post	3.35	
					BM 1year post	2.9	
4	11/23/99	SP 3783 I	395841	3/19/99	BM 112 Days post	2.65	
		SP 3783 II			BM 180 Days post	2.91	
	7/13/00	SP 3783 III			BM 335 Days post	2.6	
	9/21/00	SP 3783 IV			BM +415 Days post	2.73	
	12/28/00	SP 3783 V			BM +513 Days post	2.71	
5	2/10/00	SP 3784 I	409263	10/26/99	Cord Blood from Dono	ND	
6	3/7/00	SP 3792 I	409290	11/13/94	BM 30 days post	1.66	deceased
7	3/9/00	SP 3793 I	408771	6/21/89	BM 92 days post	2.67	
8	3/15/00	SP 3797 I	242493	11/12/86	BM 98 days post	3.66	
	Jan. 2, 2001	SP 3797 II			BM 391days post	3	
9	5/2/00	SP 3825 I	356186	5/7/88	BM 245 days post	3.08	
	7/11/00	SP 3825 II			BM 300 days post	2.56	
	8/21/00	SP 3825 III			BM 341 days post	4.56	
	9/6/00	SP 3825 IV			BM 360 days post	1.94	deceased
10	6/29/00	SP 3862 I	416805	9/24/87	BM 87 days post	3.27	
	Jan. 12,2001	SP 3862 II			BM 284 days post	3.29	
	3/19/01	SP 3862 III			Pre-transplant BM		
	4/9/01	SP 3862 IV			BM 371 days post		
11	7/25/00	SP 3874 I	418497	?	BM 92 days post	2.8	
	11/30/00	SP 3874 II			BM 219 days post	2.79	
	2/28/01	SP 3874 III			BM 308 days post	2.65	
12	7/27/00	SP 3876 I	316542	6/14/90	BM 30 days post	3.35	
	10/19/00	SP 3876 II	316542	6/14/90	BM 114 days post	2.86	
	3/12/01	SP 3876 III			BM 258 days post	2.85	
13	8/8/00	SP 3877 I	350288	8/24/95	936 Days (2.5 y) post	3.75	
14	8/16/00	SP 3878 I	408543	10/15/99	28 Days post BMT	2.98	
	10/11/00	SP 3878 II			84 Days post BMT	1.97	deceased
15	8/28/00	SP 3880 I	353947		Blood 3 years post BM	3.65	
16	8/31/00	SP 3881 I	371877	9/14/84	BM 2 years post	3.71	
17	9/22/00	SP 3882 I	422494	7/31/98	Pre-transplant BM	died	

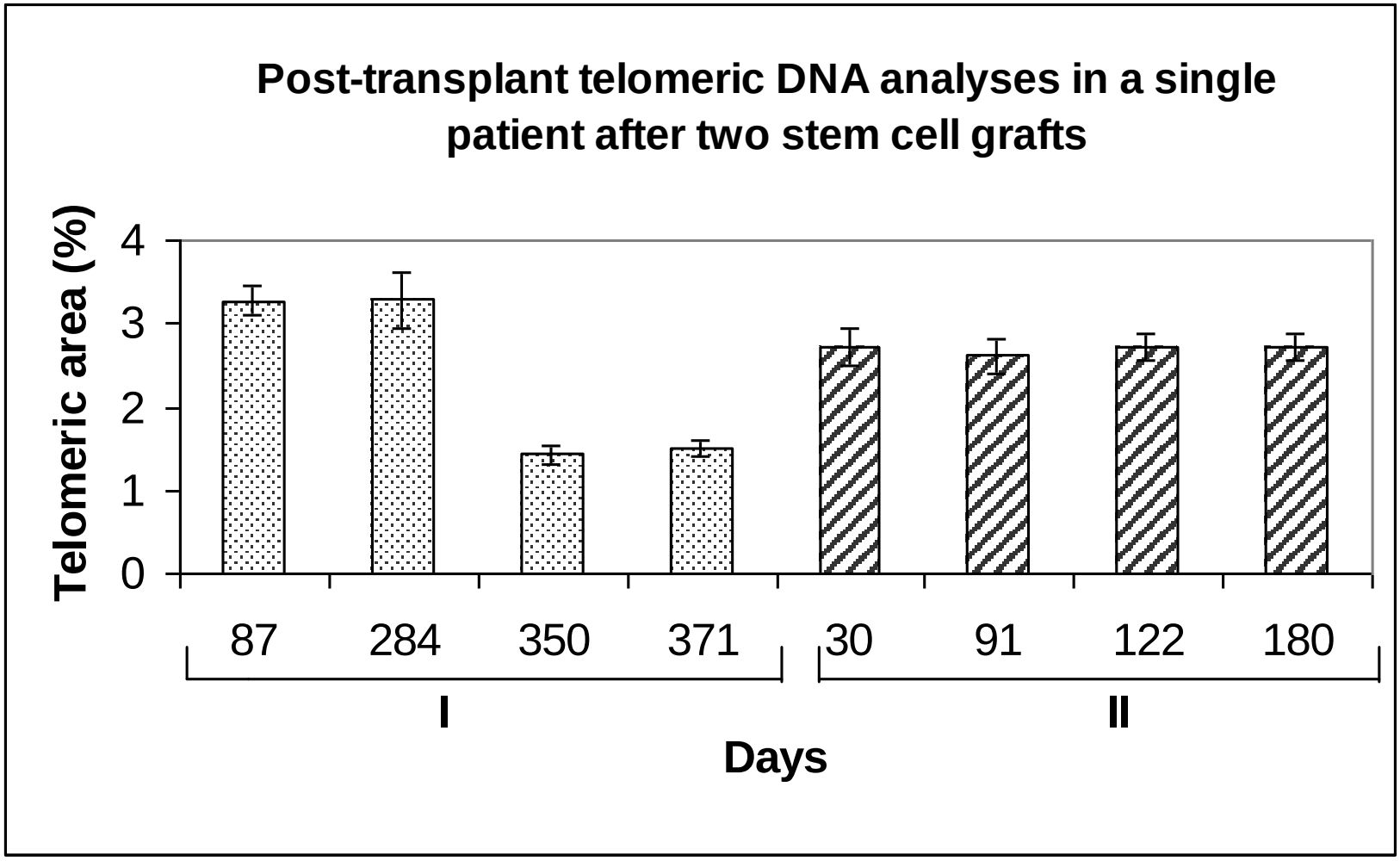
Table I. Cont'd

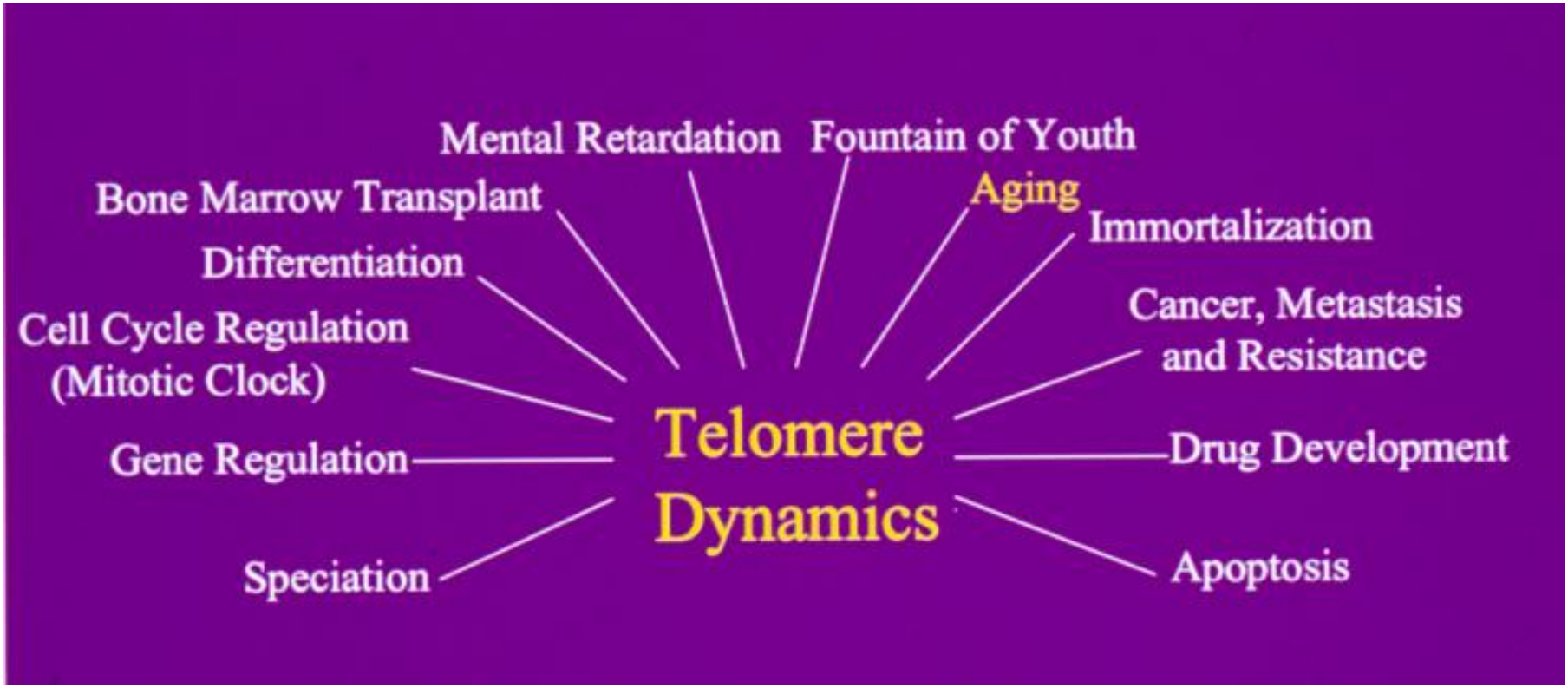
18	9/20/00	SP 3883 I	422430		Pre-transplant BM	2.74	
	11/30/00	SP 3883 II			30 Days post BMT	3.07	
	12/19/00	SP3883 III			50 Days post BMT	3.04	
	Jan. 24,2001	SP3883 IV			86 Days post BMT	2.65	
19	10/2/00	SP 3889 I	376566	7/2/91	BM 2 years post	2.49	
20	10/20/00	SP 3891 I	346759	8/8/93	Pre-transplant BM	died	
21	10/27/00	SP 3892 I	429923	4/22/85	BM 27 days post	3.25	
	12/29/00	SP 3892 II			BM 100 days post	3.26	
22	10/27/00	SP 3893 I	391776		BM 27 days post	1.34	
	11/20/00	SP 3893 II			BM 52 days post	telomere	deceased
						exclusion	
23	10/31/00	SP 3894 I	423363	4/19/92	BM 27 days post	2.92	
	Jan. 3, 2001	SP 3894 II			BM 91 days post	3.01	
24	11/20/00	SP 3907 I	407641	11/25/98	BM 97 days post	3.22	
25	12/12/00	SP 3923 I	428750	7/14/89	BM Pre-Transplant	3.28	
	Jan. 17, 200	SP 3923 II			Day 30 s/p allo BMT	2.6	
	3/29/01	SP 3923 III			BM 100 days post		
26	Jan. 4, 2001	SP3926 I	456383	9/4/98	BM Pre-Transplant	3.37	
	2/21/01	SP3926 II			30 Days post BMT	2.54	
	4/30/01	SP3926 III			BM 100 days post		
27	Jan. 10, 200	SP3927 I	381874	7/2/98	BM Pre-Transplant	2.76	
	2/21/01	SP3927 II			30 Days post BMT	2.52	
	3/28/01	SP3927 III			64 Days post BMT		
	4/30/01	SP3927 IV			BM 100 days post		
28	Jan. 12,2001	SP3928 I	456139		BM Pre-Transplant	3.27	
	3/15/01	SP3928 II			30 Days post BMT	2.22	
29	Jan. 30, 200	SP3933 I	426157	9/15/96	BM Pre-Transplant	3.22	
30	Jan. 31, 200	SP3934 I	354914	3/20/81	BM Pre-Transplant	2.27	
31	3/13/01	SP3959 I	404668	2/8/94	BM Pre-Transplant		
32	3/14/01	SP3962 I	455936	10/31/87	BM Pre-Transplant		
34	4/6/01	SP3971 I	457631	11/14/95	BM Pre-Transplant		

BONE MARROW TRANSPLANT




Post-transplant telomeric DNA analyses in 3 Patients
Telomeric area(%)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
100
200
300
400
500
Days
**
*
*
*
*
*
*
SP3874
SP3892
SP3926
** P< 0.001
* P<0.0001

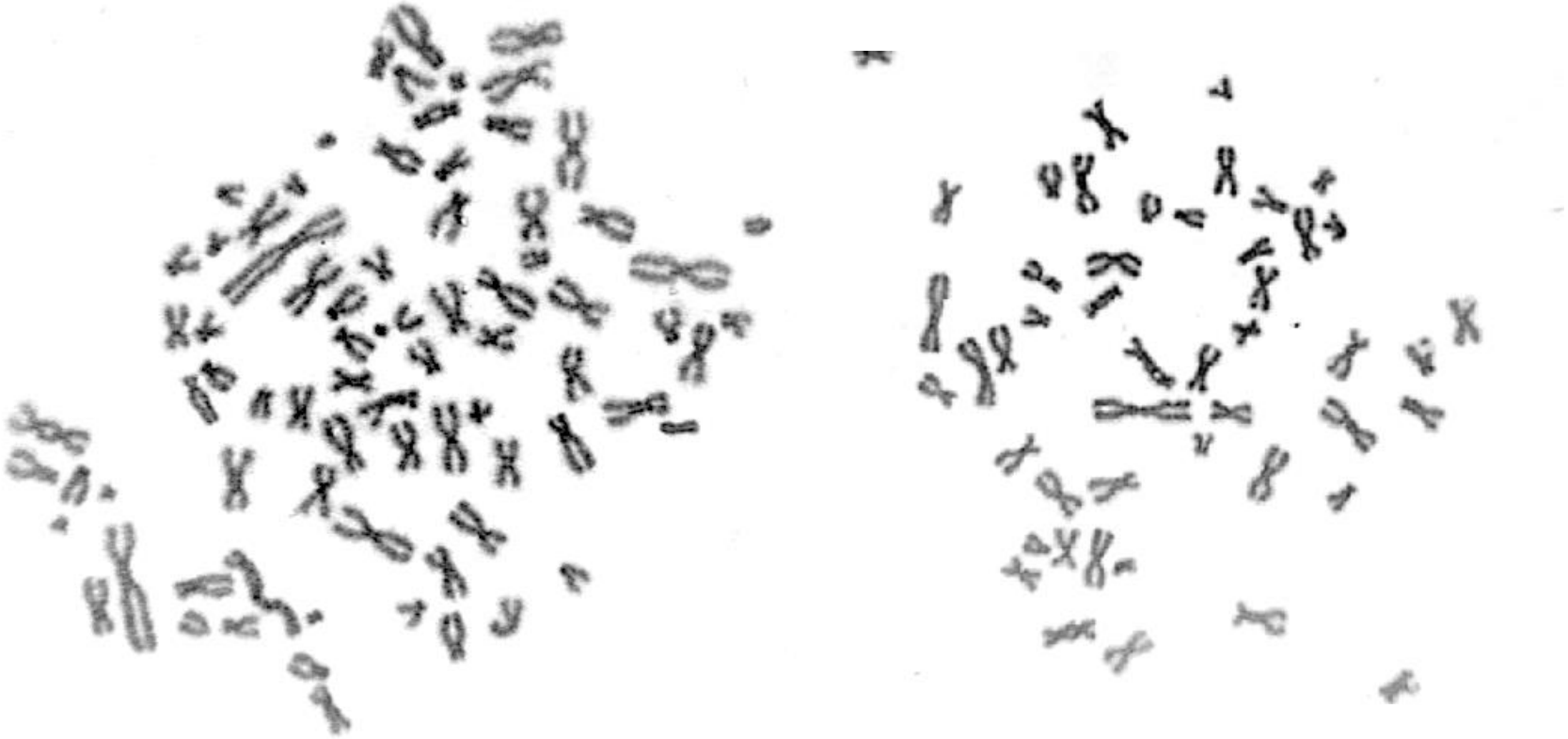

Post-transplant telomeric DNA analyses in a single patient after two stem cell grafts
Telomeric area (%)
0
1
2
3
4
87
284
350
371
30
91
122
180
I
II
Days


Mental Retardation
Fountain of Youth
Aging
Bone Marrow Transplant
Immortalization
Differentiation
Cancer, Metastasis
and Resistance
Cell Cycle Regulation
(Mitotic Clock)
Telomere
Dynamics
Gene Regulation
Drug Development
Speciation
Apoptosis

THANKS

EC Parental

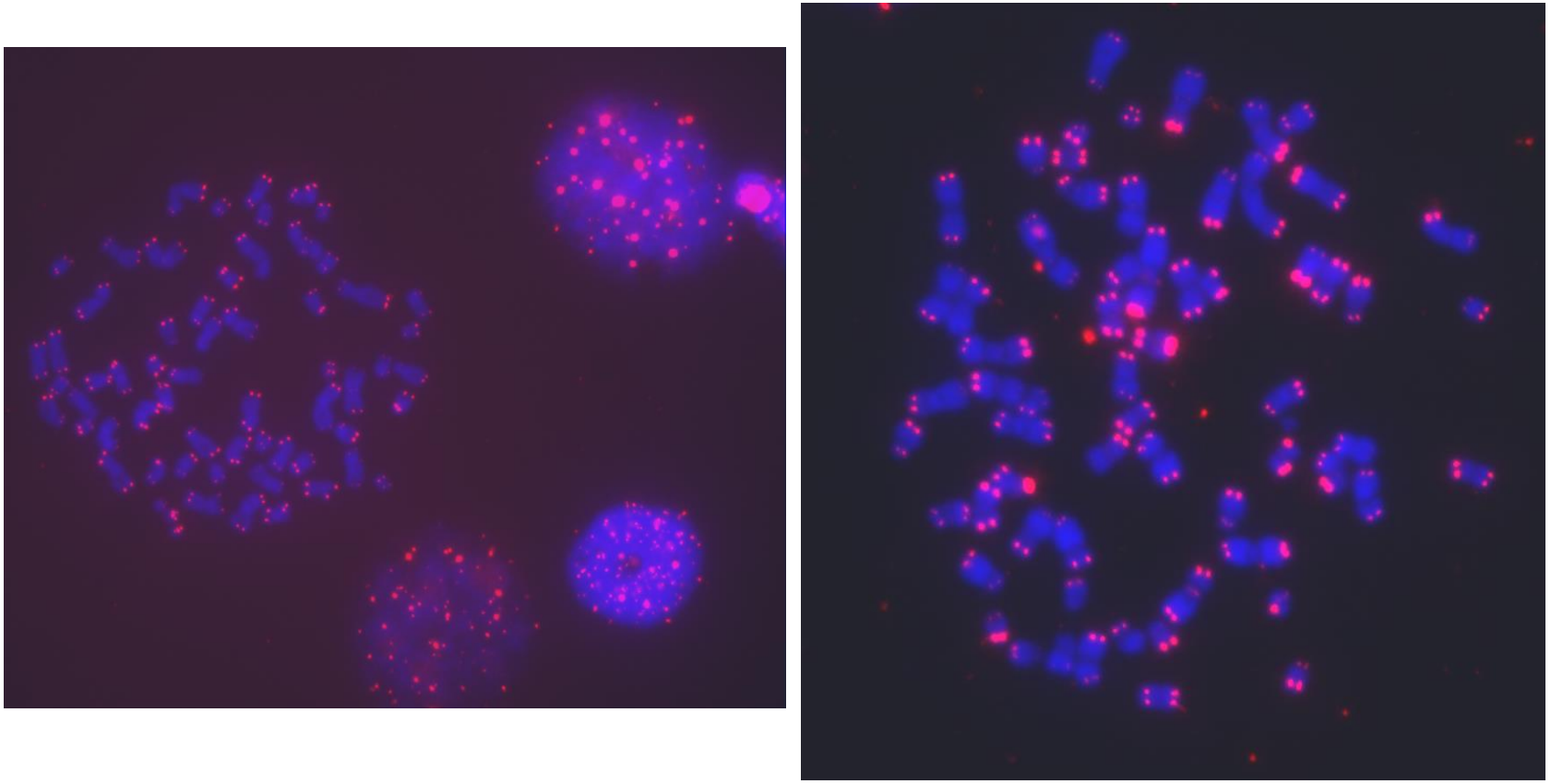
JMC Clone



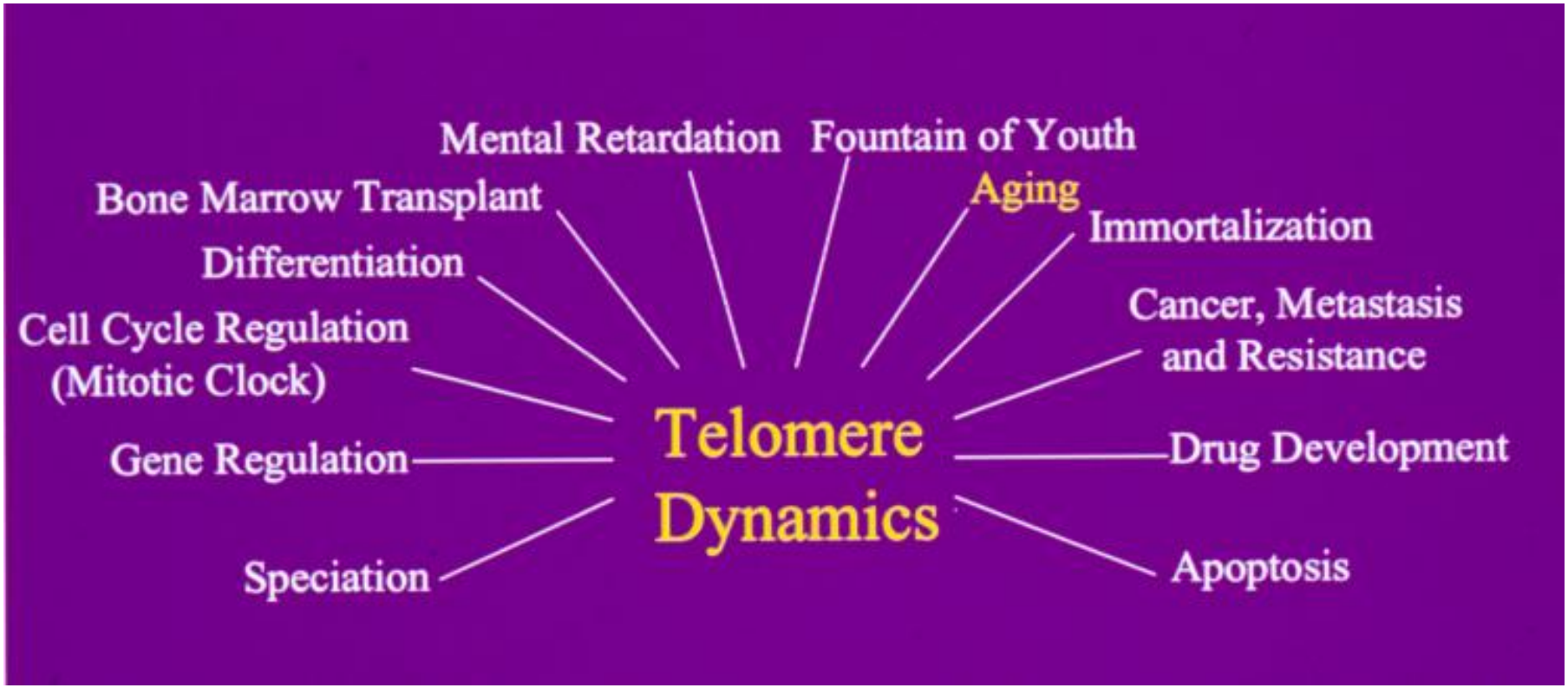
Giemsa stain

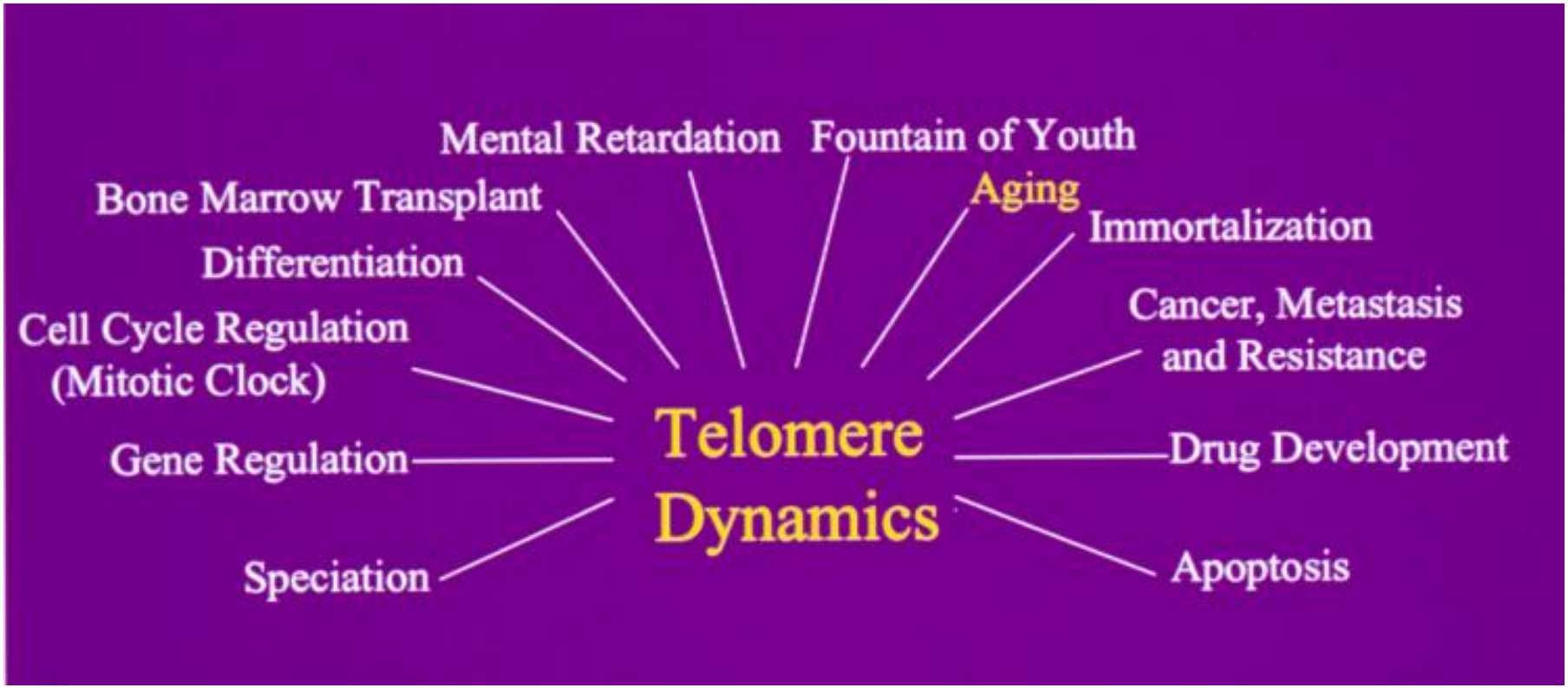
EC Parental

JMC Clone



Telomere FISH

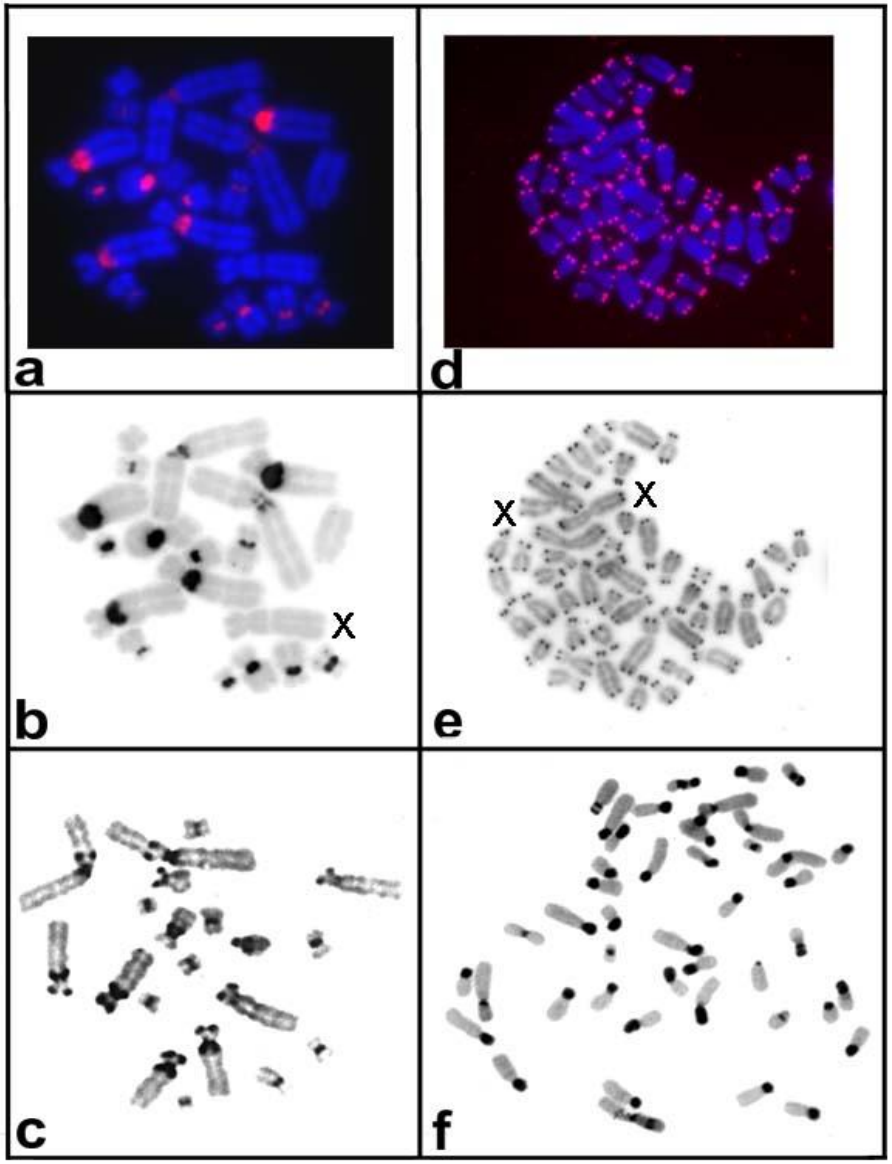

Mental Retardation
Fountain of Youth
Aging
Bone Marrow Transplant
Immortalization
Differentiation
Cancer, Metastasis
and Resistance
Cell Cycle Regulation
(Mitotic Clock)
Telomere
Dynamics
Gene Regulation
Drug Development
Speciation
Apoptosis


Mental Retardation
Fountain of Youth
Aging
Bone Marrow Transplant
Immortalization
Differentiation
Cancer, Metastasis
and Resistance
Cell Cycle Regulation
(Mitotic Clock)
Telomere
Dynamics
Gene Regulation
Drug Development
Speciation
Apoptosis

Telomere Dynamics in Cancer Cell Proliferation and Apoptosis

Sen Pathak

Department of Cancer Genetic, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030, USA


a
d
b
X
e
X
X
c
f

Pathak et al., (AMD), 1975.

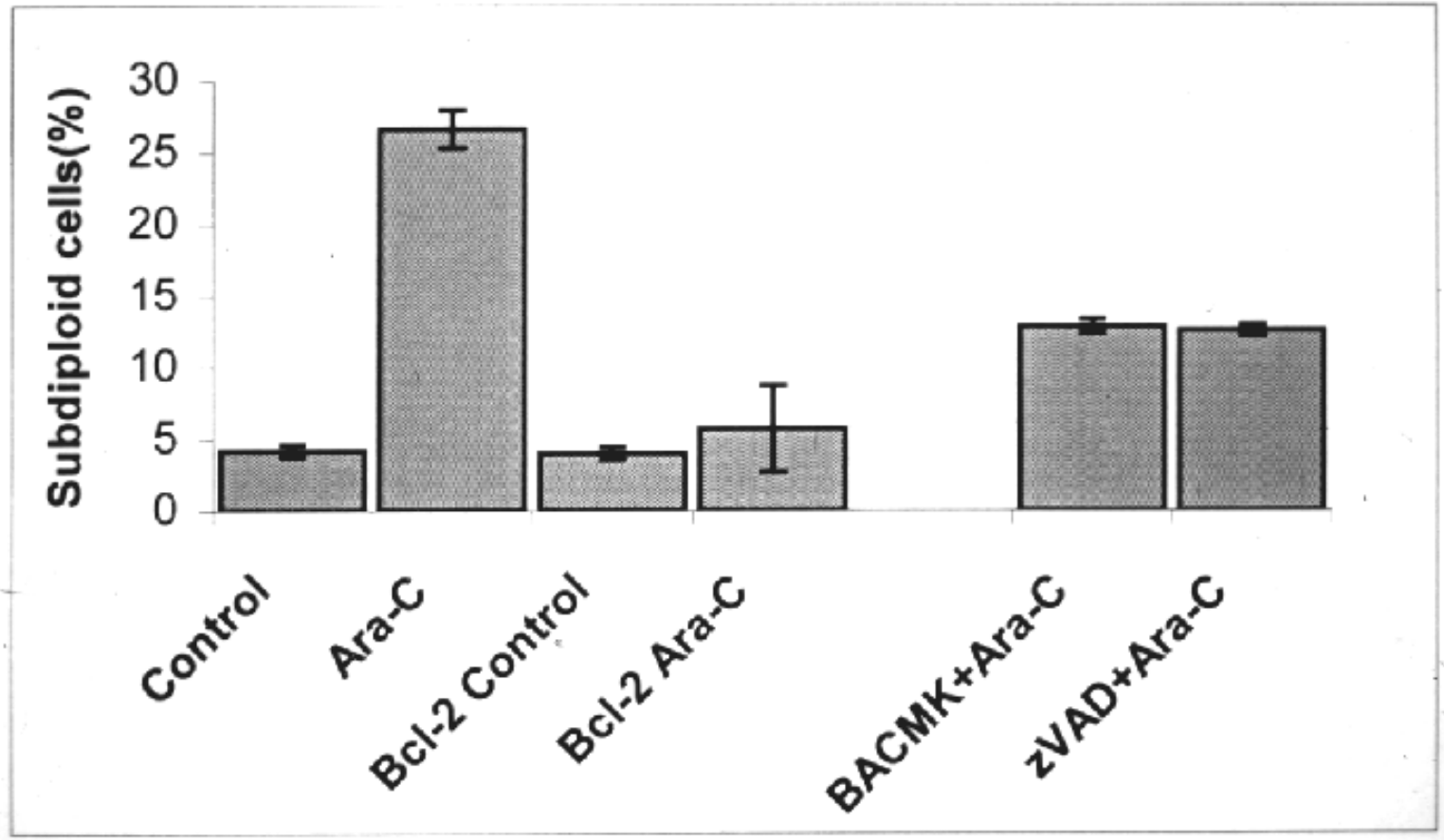
Table 1. Human telomerase components, telomere proteins, and proteins involved in the repair of telomeric DNA (tab001nkg)

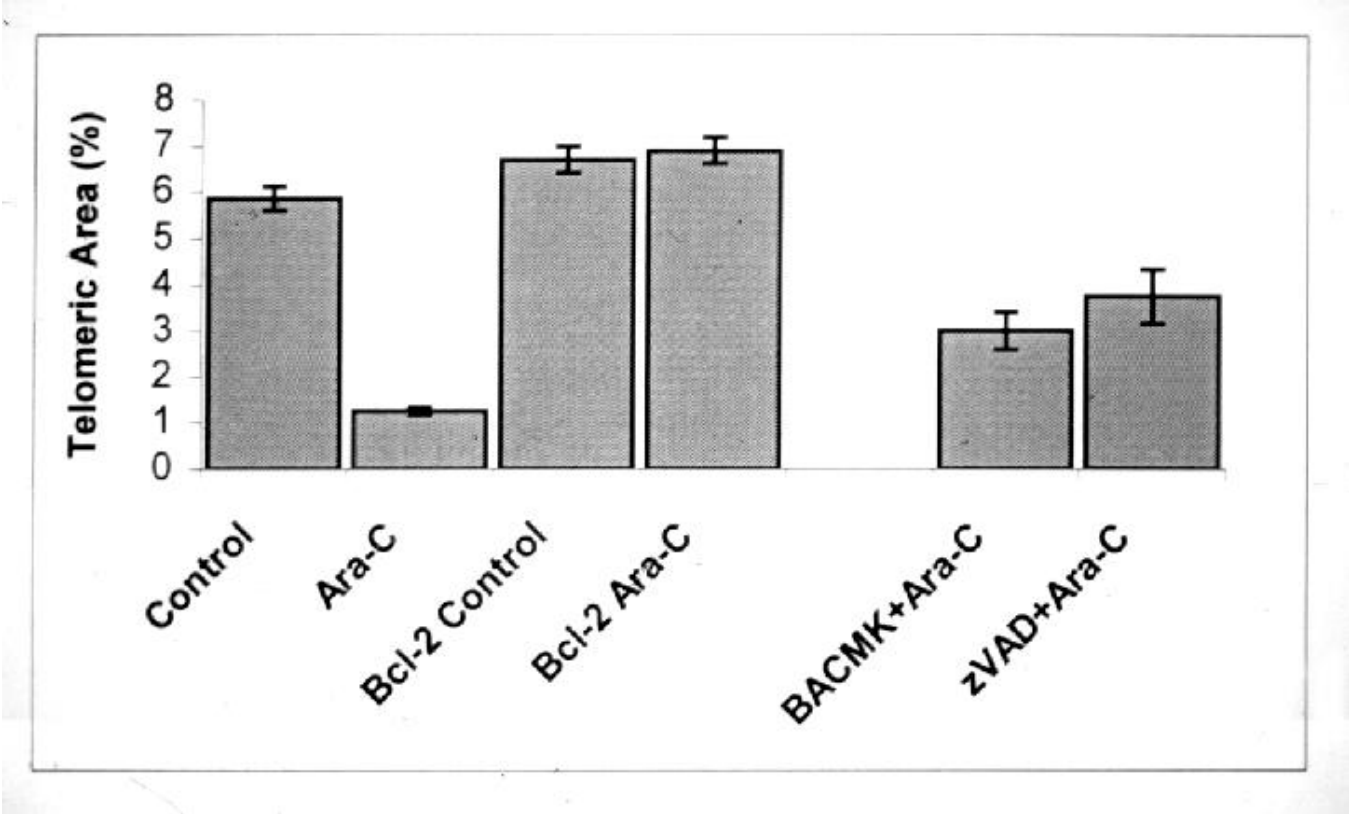
Telomerase components[a]	Description	Chromosome	Gene accession[b]	Protein accession
TERT (hTERT)	Telomerase reverse transcriptase	5p15.33	NM_003219	O14746
TERC (hTR)	Telomerase RNA component	3q26.3	HSU86046	
HSPCA (HSP90)	Heat shock 90 kDa protein 1, alpha	1q21.2-q22	NM_005348	NP_005339
P23	Telomerase-binding protein, p23	12	XM_006707	Q15185
TEP1 (TP1)	Telomerase-associated protein 1	14q11.2	NM_007110	XP_007488
SSB (La)	Sjogren syndrome antigen B (autoantigen La)	2p14-q14.3	NM_003142	NP_003133
RPL22 (L22)	Ribosomal protein L22	3q26	NM_000983	NP_000974
STAU	Staufen (*Drosophila* RNA-binding protein)	20q13.1	XM_016758	O95793
DKC1	Dyskeratosis congenita 1, dyskerin	Xq28	XM_053357	NP_001354
NOLA1 (GAR1)	Nucleolar protein family A, member 1 (H/ACA small nucleolar ribonucleoproteins)	4q	XM_054788	NP_127460
Telomere proteins				
TERF1 (TRF1)	Telomeric-repeat-binding factor (NIMA-interacting) 1	8q13	XM_016344	XP_016344
TERF2 (TRF2)	Telomeric-repeat-binding factor 2	16q22.1	XM_028687	XP_028687
TNKS (tankyrase)	Tankyrase, TRF1-interacting ankyrin-related (ADP-ribose)polymerase	8q	NM_003747	NP_003738
TNKS2 (TANK2)	Tankyrase, TRF1-interacting ankyrin-related (ADP-ribose)polymerase 2	10q23.3	NM_025235	NP_079511
TINF2 (TIN2)	TERF1 (TRF1)-interacting nuclear factor 2	14q12-14q21.3	XM_033252	XP_007309
RAP1	TRF2-interacting telomeric RAP1 protein	16	XM_033974	XP_033974
POT1	*Homo sapiens* cDNA FLJ11073, putative telomere-end-binding protein		AK001935	
WRN	Werner syndrome (control of genomic stability)	8p12-p11.2	NM_000553	Q14191
ADPRT (PARP)	ADP-ribosyltransferase [NAD^+; poly (ADP-ribose) polymerase]	1q41-q42	NM_001618	P09874
Telomere repair				
MRE11A	Meiotic recombination (*Saccharomyces cerevisiae*) 11 homologue A	11q21	XM_045811	P49959
NBS1	Nijmegen breakage syndrome 1 (nibrin)	8q21	NM_002485	NP_002476
RAD50	Rad50 (*S. cerevisiae*) homologue	5q31	NM_005732	XP_034865
G22P1 (KU70)	Thyroid autoantigen 70 kDa (Ku antigen)	22q13.2-q13.31	NM_001469	P12956
XRCC5 (KU80)	X-ray repair (double-strand-break rejoining; Ku autoantigen, 80 kDa)	2q35	M30938	P13010
PRKDC (DNAPK)	Protein kinase, DNA-activated, catalytic polypeptide (DNA-PKCS) (DNPK1)	8q11	NM_006904	P78527
ATM	Ataxia telangiectasia; involved in signal transduction, cell cycle control and DNA repair	11q22-q23	NM_000051	Q13315

Human telomerase components, telomere proteins, and proteins involved in the repair of telomeric DNA

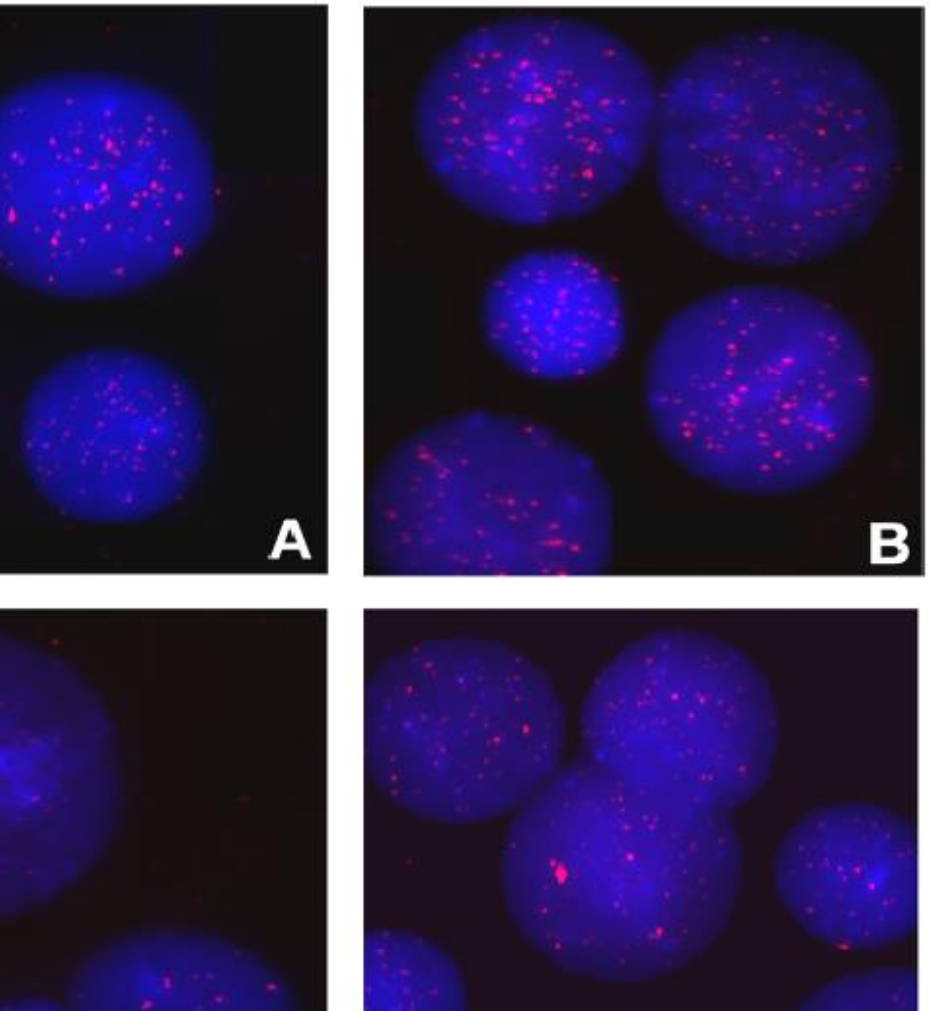
Mechanisms of Telomere Length Maintenance

- **Telomerase-mediated; common in cancer cells, slow process, less heterogeneity in telomeres**
- **Recombination-mediated or Alternative lengthening of telomere (ALT); rare in cancer, sudden increase, great heterogeneity of telomere size (ranging from undetectable to abnormally long) within individual cells, presence of nuclear bodies containing extrachromosomal telomeric DNA**
- **Both mechanisms operating simultaneously in some tumors**

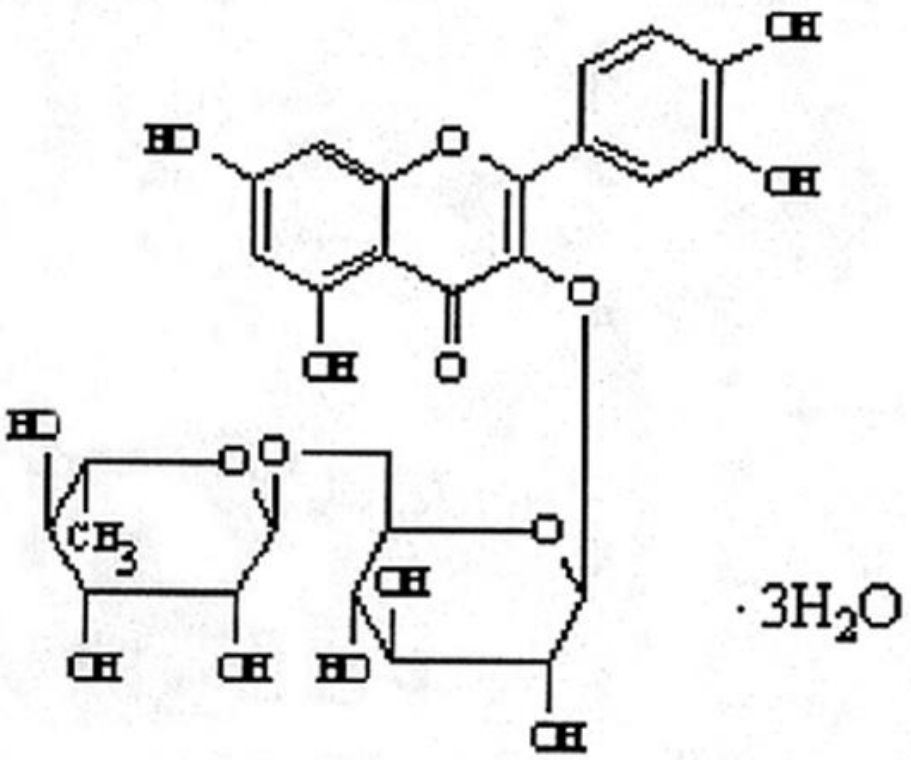

Subdiploid cells(%)
30
25
20
15
10
5
0
Control
Ara-C
Bcl-2 Control
Bcl-2 Ara-C
BACMK+Ara-C
zVAD+Ara-C


Telomeric Area (%)
8
7
6
5
4
3
2
1
0
Control
Ara-C
Bcl-2 Control
Bcl-2 Ara-C
BACMK+Ara-C
zVAD+Ara-C

Azoospermia



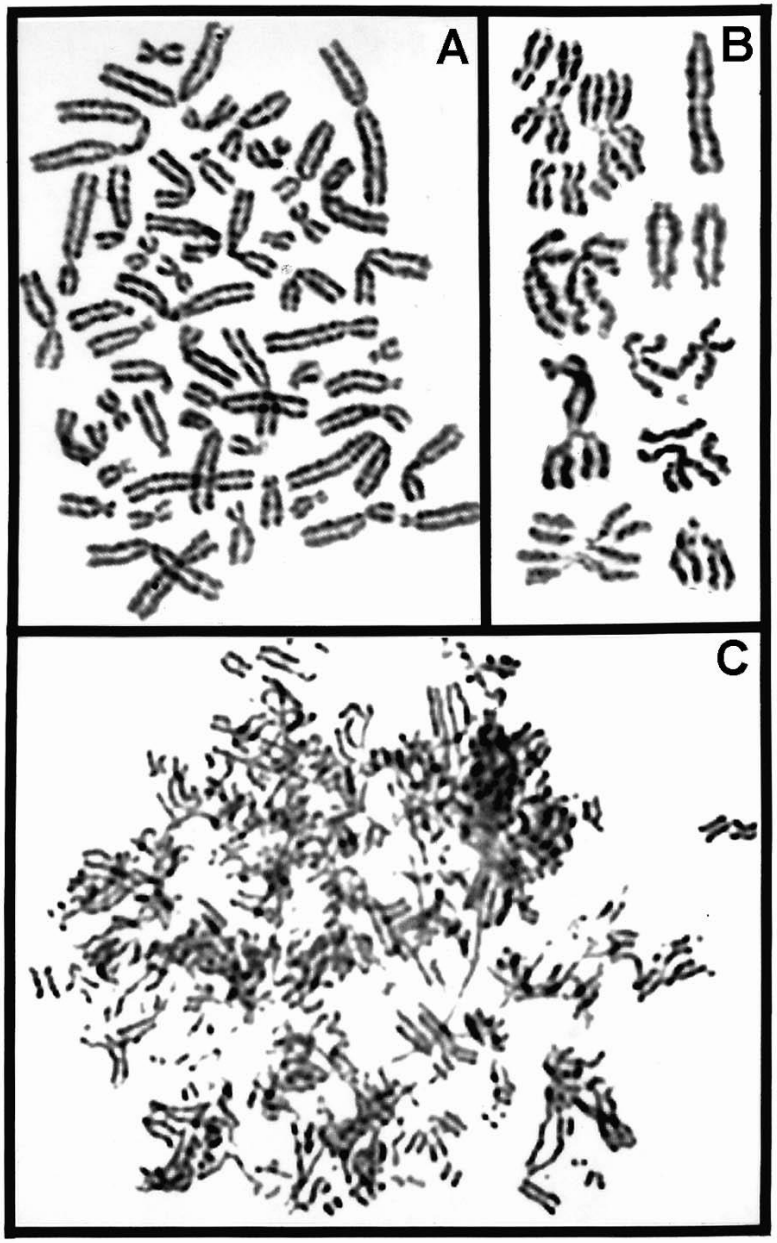
Ruta Structure



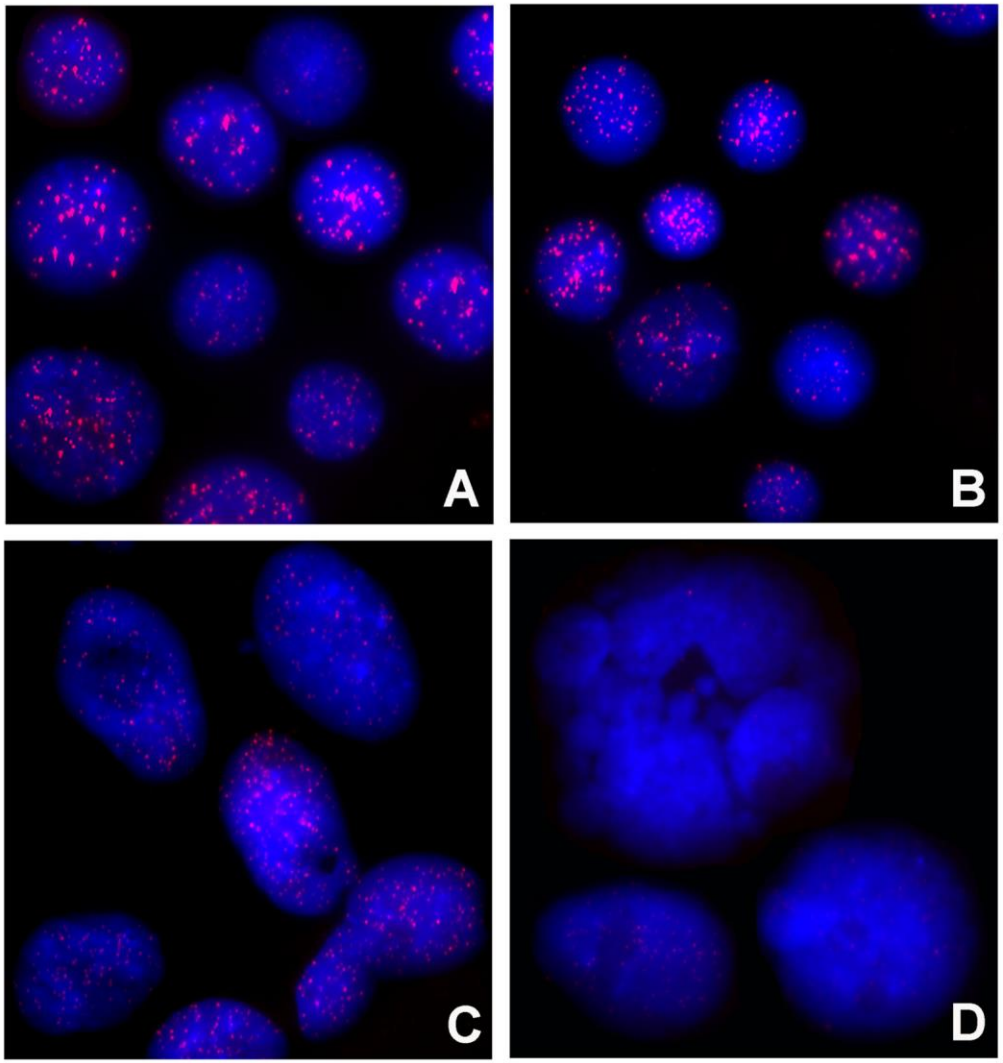
FORMULA: $C_{27}H_{30}O_{16} \cdot 3H_2O$

MOL. WEIGHT: 664,57

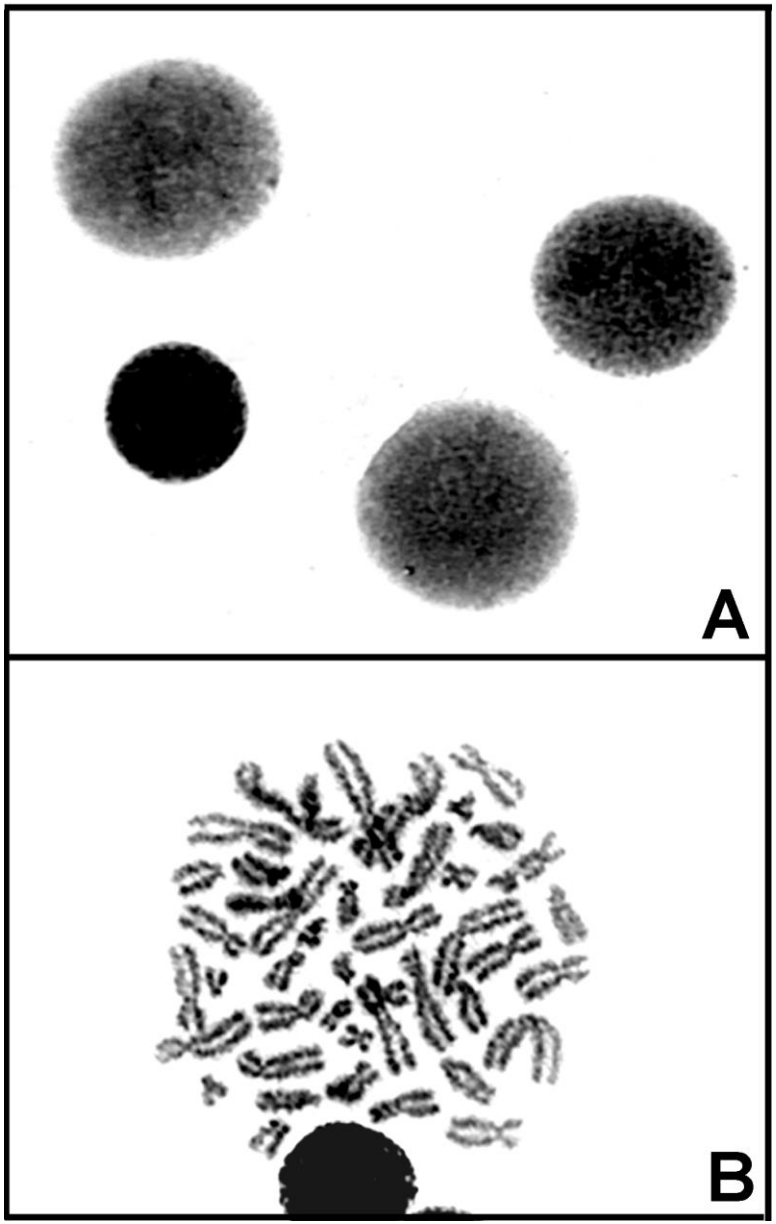
Ruta treatment on GBM



Ruta treatment



Ruta and PBL



Ruta treatment

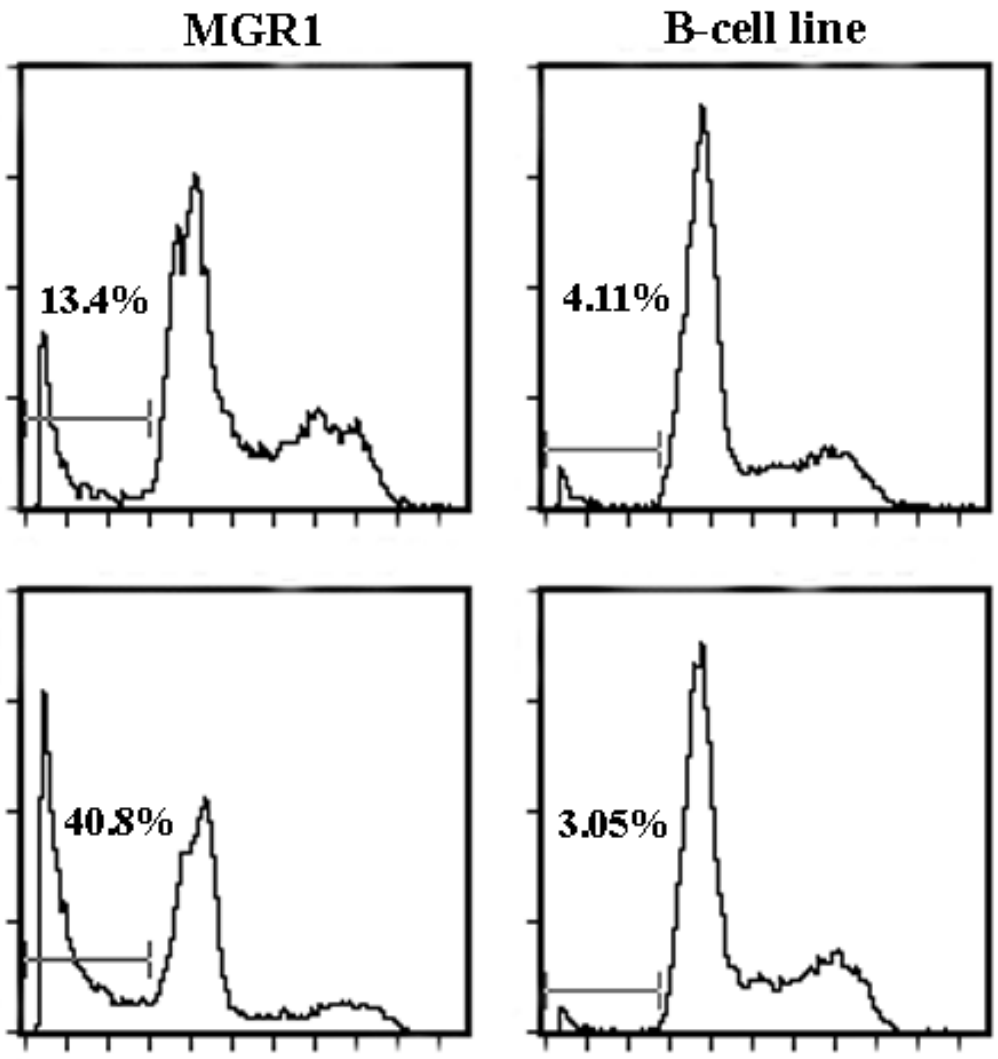


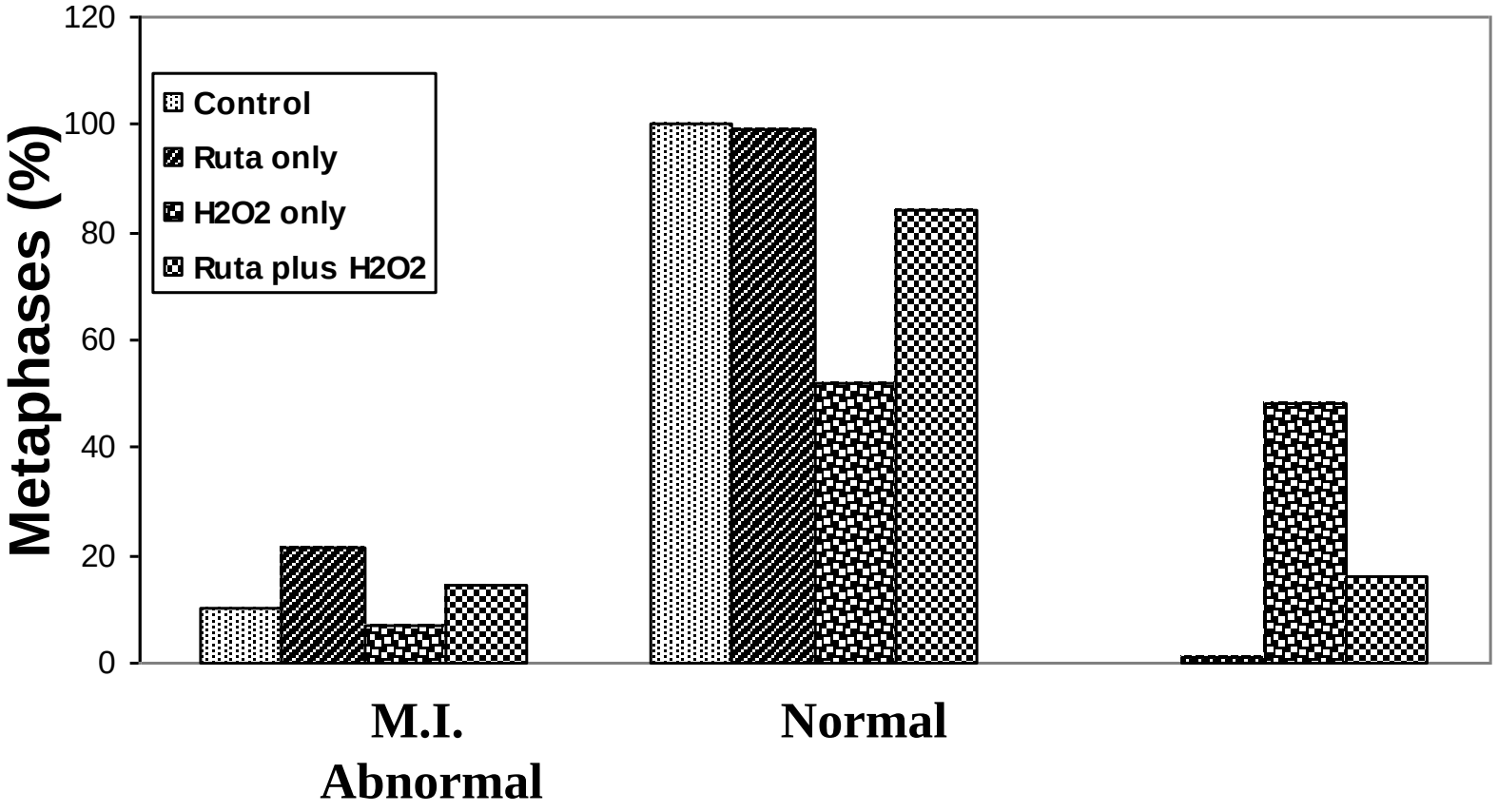
Table 2: Frequency of normal and abnormal metaphases in Ruta 6 + $CaPO_4$ -treated human brain cancer cells

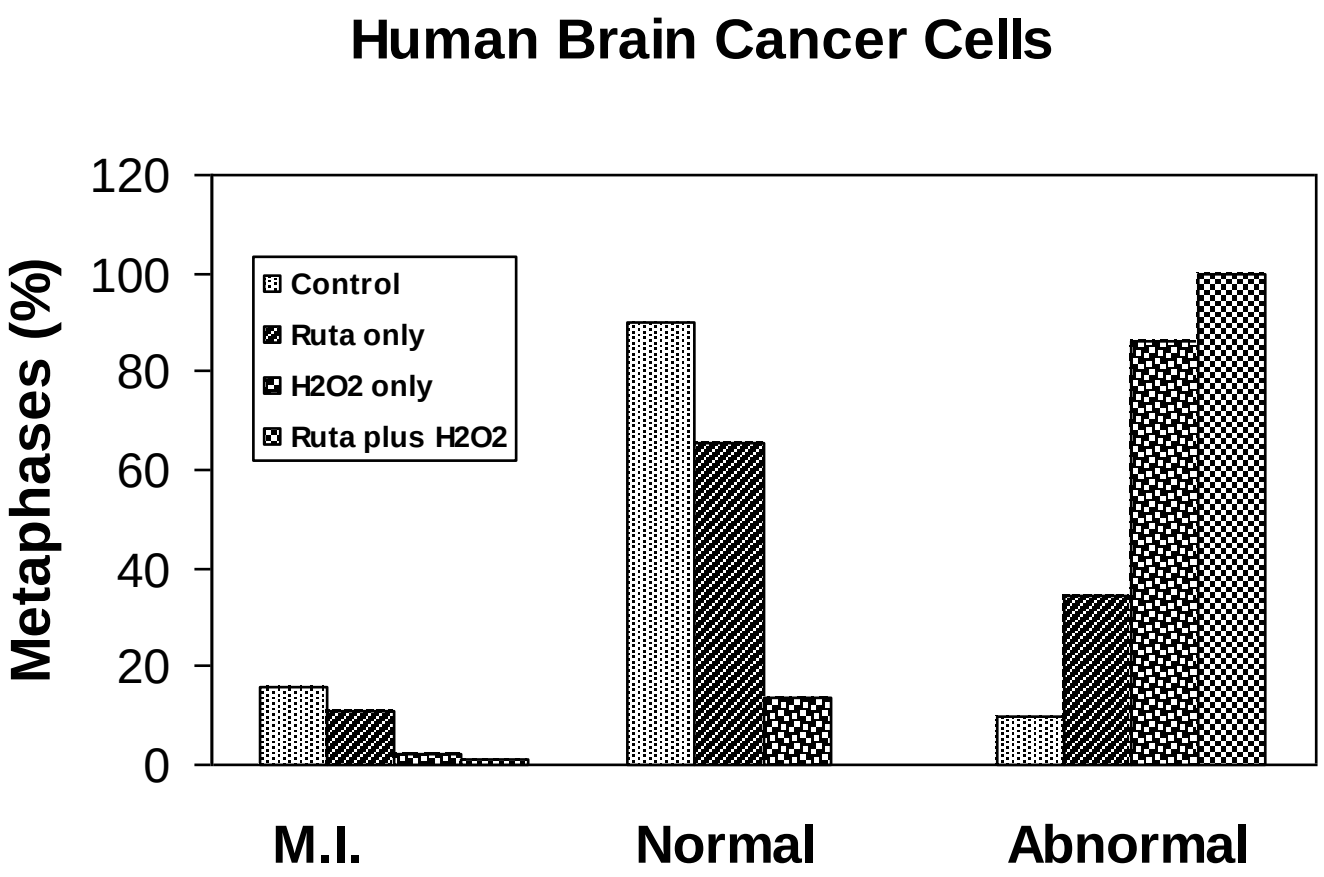
Experiment Number	Dose	Mitotic Index (%)	Duration (hours)	Normal Metaphases % 1S	% 2S	% Metaphases with Aberrations
SP4262	Control	15.8	24	90.2	2.0	8.0
SP4363	Ruta 6 (Low dose)	10.3	24	42.8	7.6	49.5
SP4364	Ruta 6 (High dose)	9.6	24	30.7	4.9	64.3
SP4267	Ruta 1	12.2	24	22.8	1.3	75.9
SP4293	Ruta 0	0.9	24	0.0	0.0	100.0

Table 1: Frequency of metaphases with aberrations in a B-lymphoid cell line treated for 24 hr with Ruta + $CaPO_4$

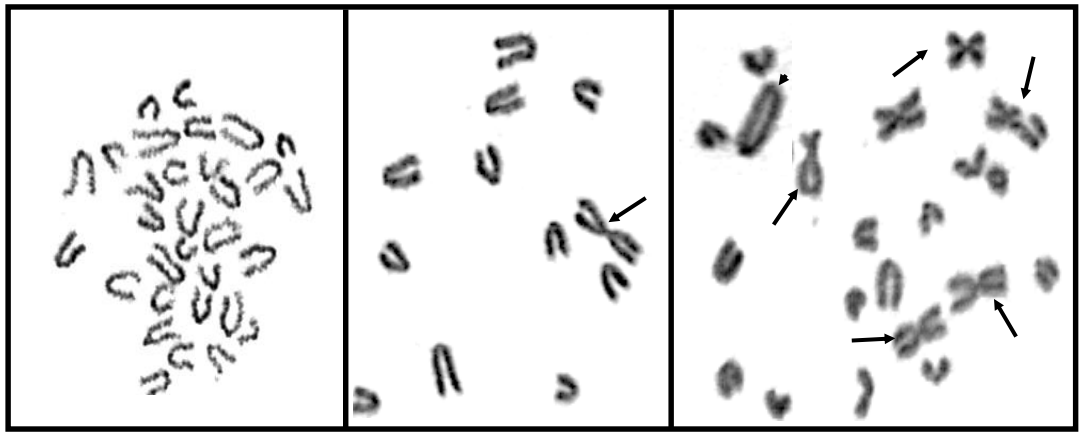
Experiment Number	Dose	Normal Metaphases % 1S	% 2S	% Metaphases with Aberrations
SP4338	Control	92.0	8.0	0.0
SP4341	Ruta 6	91.4	3.8	4.8
SP4345	0.2 µ M H_2O_2	46.0	6.0	48.0
SP4342	Ruta 6 + 0.2 µ M H_2O_2	79.0	2.0	9.0
SP4343	Ruta 1	91.2	4.9	3.9
SP4344	Ruta 1 + 0.2 µ M H_2O_2	87.4	3.9	8.7

Normal Human B-Lymphoid Cells

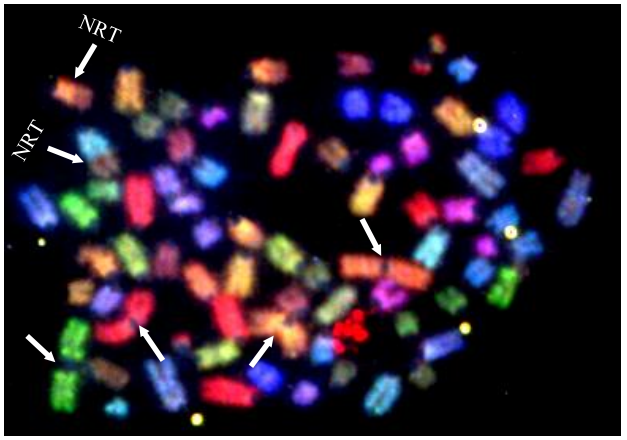



Human Brain Cancer Cells
Metaphases (%)
120
100
80
60
40
20
0
Control
Ruta only
H2O2 only
Ruta plus H2O2
M.I.
Normal
Abnormal

Mouse embryonic fibroblasts in culture



Parental P1 Parental P4 Immortalized P1



SKY

G5 mTerc-/- wrn-/- MEFs exhibiit chromosomal aberrations

Dysfunctional telomeres required for WS Pathogenesis

Human WS	Wrn-/-	G4-6 Wrn+/+	G4-6 Wrn-/-
Osteoperosis	No	+/-	++++
Cataracts	No	No	++++
Type II Diabetes	No	No	++++
Skin defects	No	++	++++
Hypogonadism	No	++	++++
Atherosclerosis	No	No	No
Genome Instability	No	++	++++
Mesenchymal tumors	+	+	++++

Acknowledgements

MDACC

Asha S. Multani
Sandy Chang

Noelia Cabrera

Purnima Laud

Kumar Ganesan

DFCI/HMS

Ron DePinho

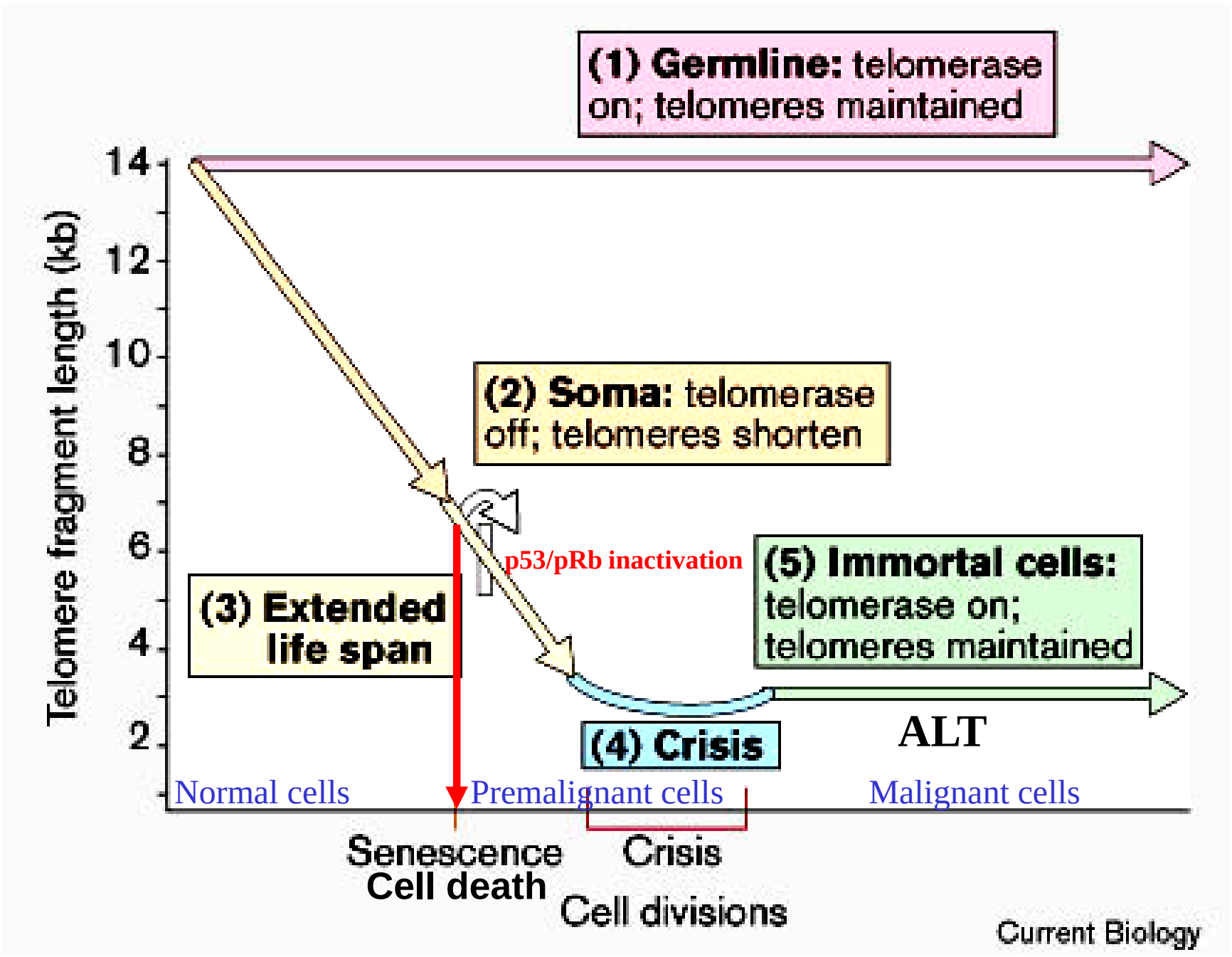
Maria Naylor

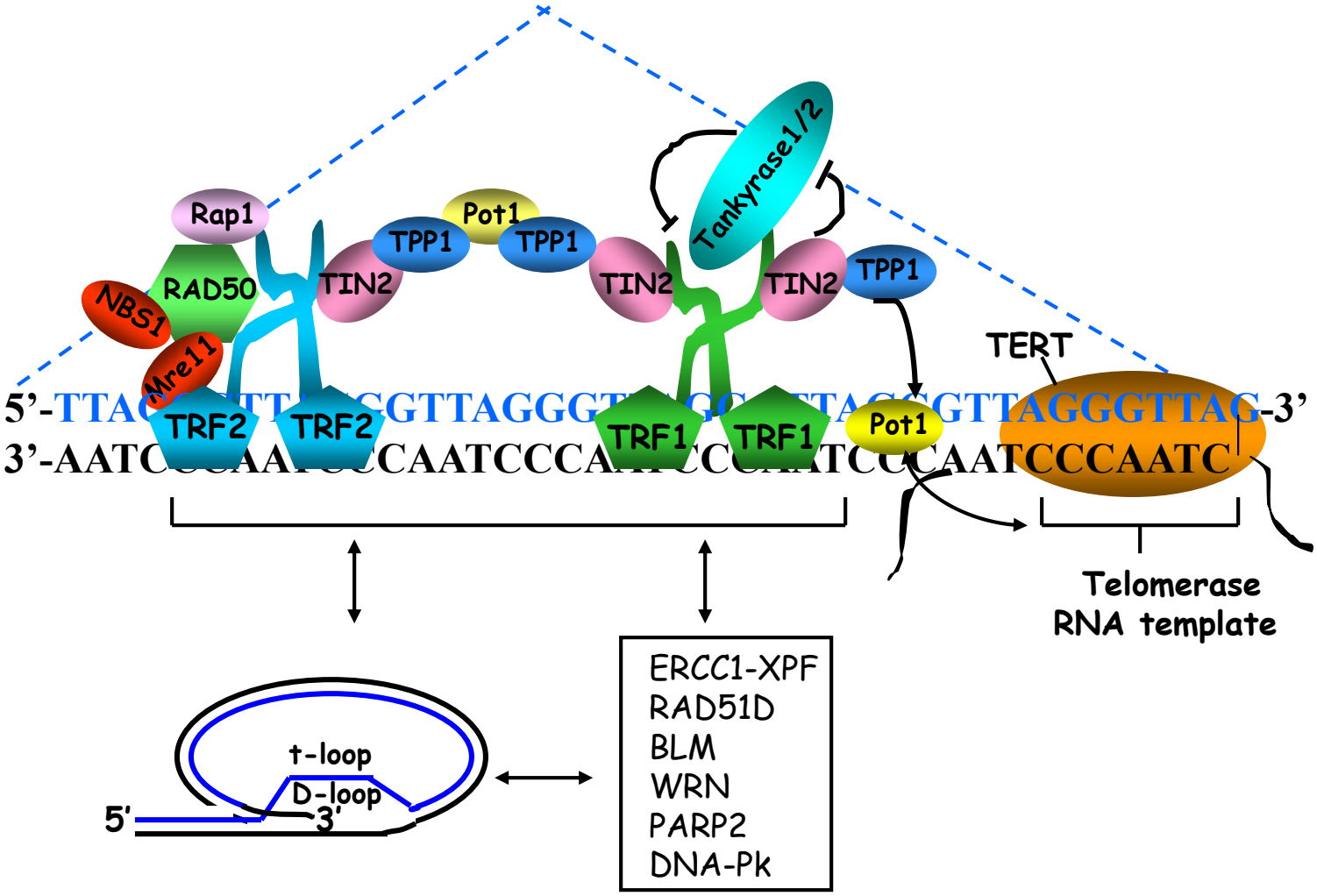
MIT

Leonard Guarente

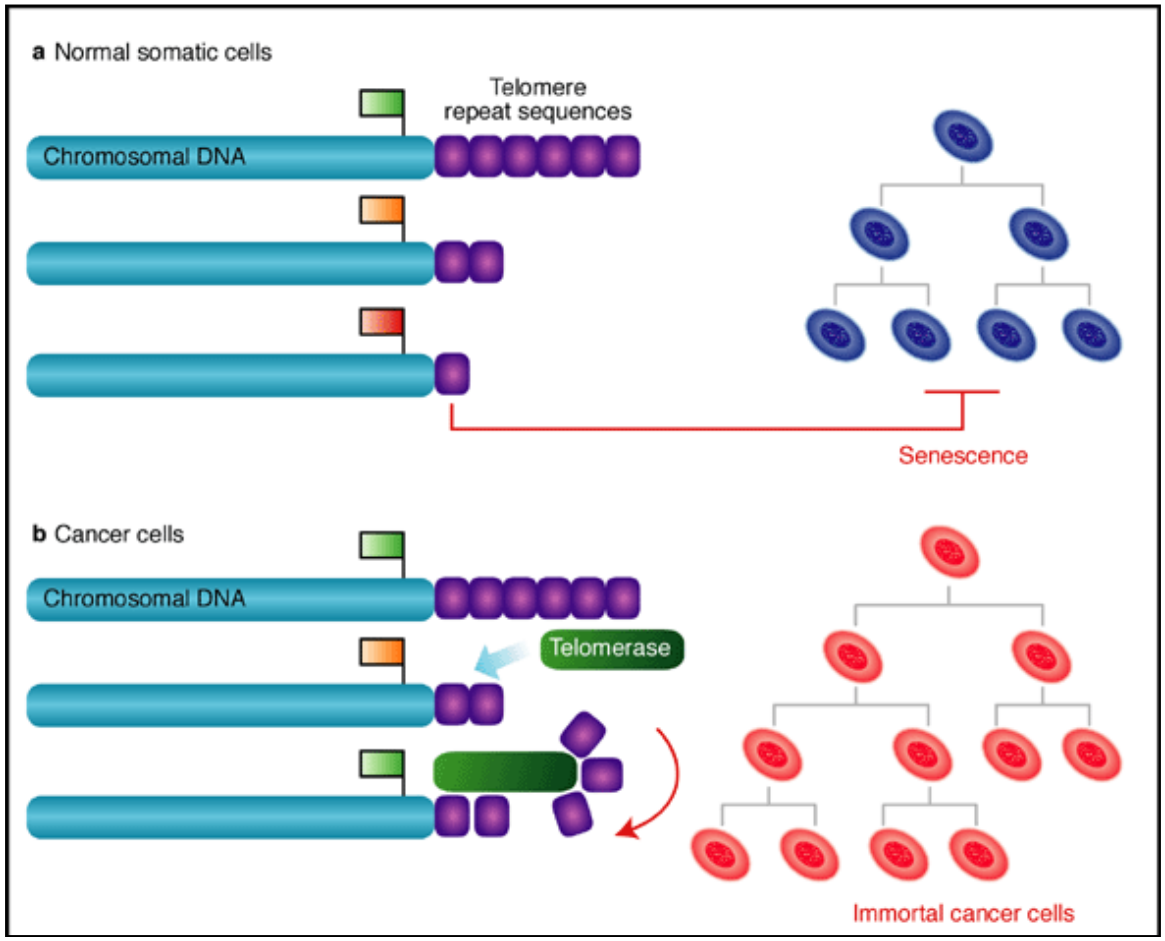
NIA

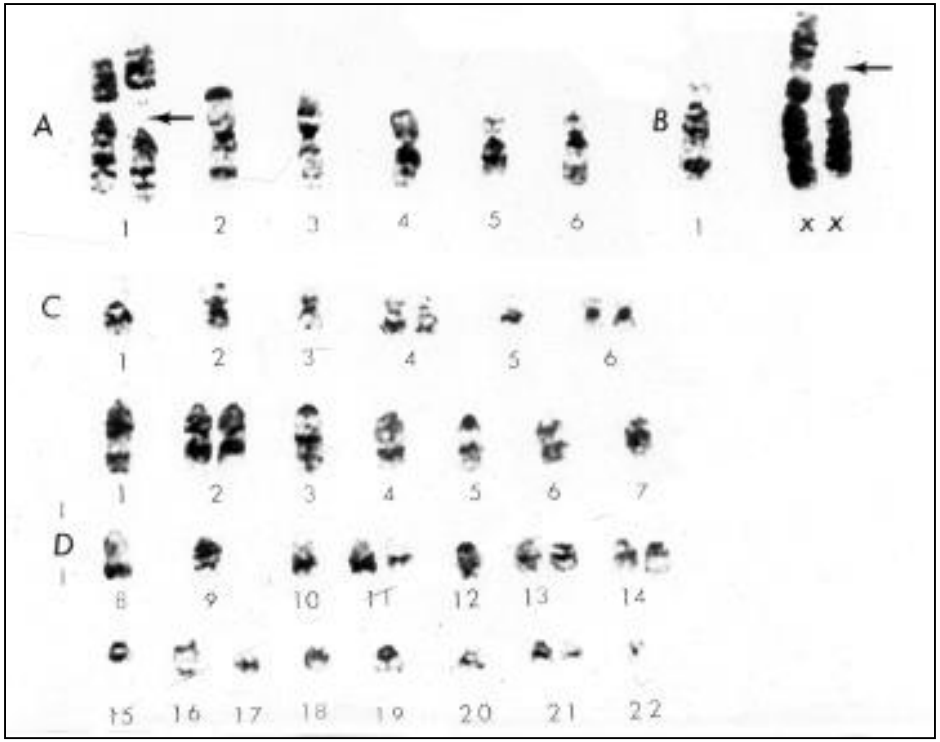
Ellison Medical Foundation


(1) Germline: telomerase on; telomeres maintained
(2) Soma: telomerase off; telomeres shorten
(3) Extended life span
p53/pRb inactivation
(4) Crisis
(5) Immortal cells: telomerase on; telomeres maintained
ALT
Telomere fragment length (kb)
14
12
10
8
6
4
2
Normal cells
Premalignant cells
Malignant cells
Senescence
Cell death
Crisis
Cell divisions
Current Biology


Rap1
RAD50
NBS1
Mre11
TRF2
TRF2
TIN2
TPP1
Pot1
TPP1
TIN2
Tankyrase1/2
TIN2
TPP1
TERT
5'-TTAGGGTTAGGGTTAGGGTTAGGGTTAG-3'
3'-AATCCCAATCCCAATCCCAATCCCAATCCCAATC
TRF1
TRF1
Pot1
Telomerase
RNA template
t-loop
D-loop
5'
3'
ERCC1-XPF
RAD51D
BLM
WRN
PARP2
DNA-Pk

Regulation of telomere length in normal and cancer cells by telomerase




A
1
2
3
4
5
6
B
1
X X
C
1
2
3
4
5
6
1
2
3
4
5
6
7
D
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22