# Disruption of Telomere Equilibrium Sensitises Human Cancer Cells to DNA Repair Inhibition and Radiation

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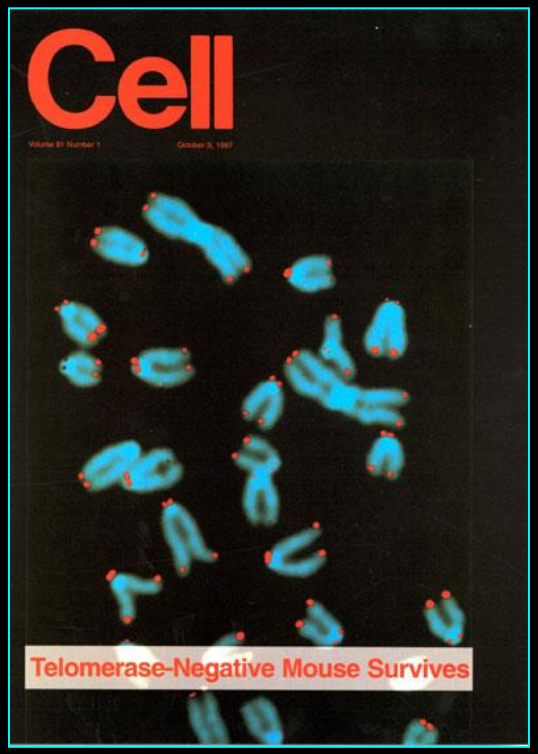
# Absence of Telomerase in Mice




Blasco, Lee, Hande, Samper, Lansdorp, DePinho, Greider 1997, *Cell* 91:25-34

- Telomere length dynamics in telomerase negative mouse embryonic fibroblasts (Hande *et al*. 1999 *J. Cell Biol*.144: 589-601)

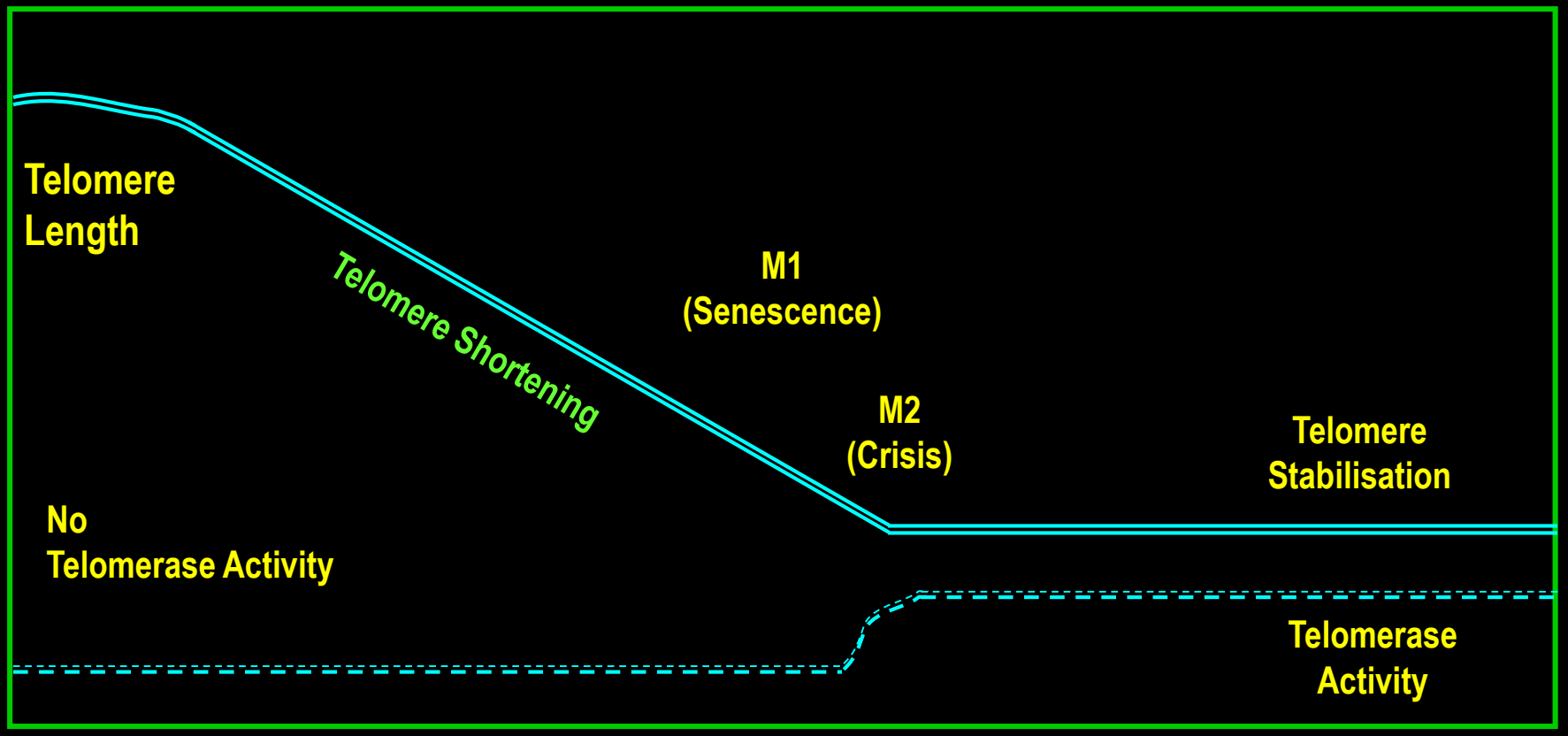
## Role of DNA repair factors in telomere maintenance

- Altered telomere homeostasis and metabolism – shortened telomeres (d'Adda di Fagagna, Hande et al. 1999, *Nature Genetics* and 2001 *Current Biology*; Hande et al. 1999 *Genomics*; Hande et al. 2001 *Human Molecular Genetics*; Hsu, Gilley, Galande, Hande et al. 2000 *Genes and Development*; McPherson, Hande et al. 2006 *Human Molecular Genetics*)
- Heightened genomic instability – chromosome fusions
- Sensitivity to ionising radiation

Hande (2004) ***Cytogenet Genome Res*** 104:116-122

Low and Hande (2008) ***ICCB 2008 Proceedings*** pp 113-20

# Telomere/Telomerase Model of Ageing and Cancer




Adapted from Shay and Wright 1999

# Telomerase activity in human cancers

| Pathology | % Positive (range) |
|---|---|
| Normal or adjacent to malignancy | 15.5 % (0 – 100%) |
| Pre-invasive cancer | 29.5% (0 – 67%) |
| Malignant | 95% (8 – 100%) |

**Recent estimate : 85-90%**

Shay and Wright 2005 Cancer Cell , 7: 1-2

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## Thymoquinone Induces Telomere Shortening, DNA Damage and Apoptosis in Human Glioblastoma Cells

**Resham Lal Gurung[1], Shi Ni Lim[1], Aik Kia Khaw[1], Jasmine Fen Fen Soon[1], Kirthan Shenoy[1], Safiyya Mohamed Ali[1], Manikandan Jayapal[1], Swaminathan Sethu[1], Rajamanickam Baskar[2], M. Prakash Hande[1]***

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*GENES, CHROMOSOMES & CANCER 51:961–974 (2012)*

## Genistein Induces Growth Arrest and Suppresses Telomerase Activity in Brain Tumor Cells

**Aik Kia Khaw,[1] Jacklyn Wei Yan Yong,[1] Guruprasad Kalthur,[1,2] and M. Prakash Hande[1,3]***

[1]Department of Physiology, Genome Stability Laboratory, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Republic of Singapore
[2]Department of Obstetrics and Gynaecology, Clinical Embryology, Kasturba Medical College, Manipal University, Manipal 576 104, India
[3]Tembusu College, National University of Singapore, Singapore 138598, Republic of Singapore

## MST-312 Alters Telomere Dynamics, Gene Expression Profiles and Growth in Human Breast Cancer Cells

Resham Lal Gurung[a] Shi Ni Lim[a] Grace Kah Mun Low[a]
M. Prakash Hande[a, b]

J Nutrigenet Nutrigenomics 2014;7:283–298

Gurung *et al. Molecular Cancer* 2014, **13**:232
http://www.molecular-cancer.com/content/13/1/232

MOLECULAR CANCER

RESEARCH Open Access

## Targeting DNA-PKcs and telomerase in brain tumour cells

Resham Lal Gurung[1,3], Hui Kheng Lim[1], Shriram Venkatesan[1], Phoebe Su Wen Lee[1] and M Prakash Hande[1,2*]

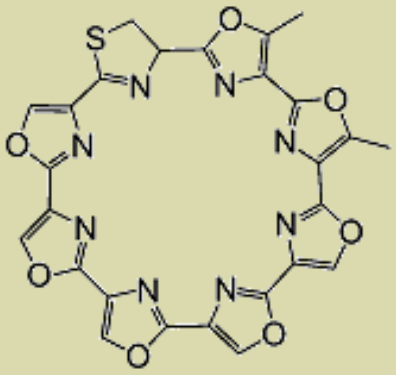
MST-312: Synthetic telomerase inhibitor*
Tea catechin (epigallocatechin gallate, EGCG
NU7026 – DNA-PKcs inhibitor

Combined inhibition of PARP-1 and Telomerase decreases cell viability in breast cancer cells - Gurung et al. (2014) J. Nutrigenet Nutrigenomics 7: 283 - 298)

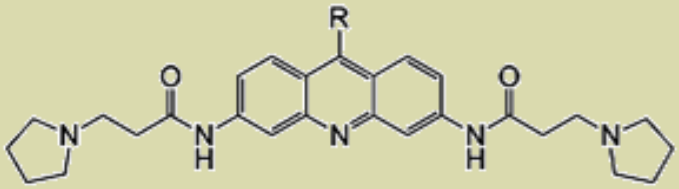
# G-quadruplex (G4) Structures

- They do exist *in vivo* and in human cells (Biffi et al., 2013)
- Predominantly form at the telomeres due to the availability of long tracts of G-rich DNA, dynamic nature of the telomere and favourable energy stabilisiation (Huppert and Balasubramanian 2005, Todd et al. 2005, Huppert 2015, Lipps and Rhodes 2009)
- About 40% of the promoters have at least one potential G4 (PG4) (Huppert and Balasubramanian 2007)
- Tumour suppressor genes are associated with low occurrence of PG4s, whereas protooncogenes correlate to a high occurrence of PG4s (Eddy and Maizels 2006)

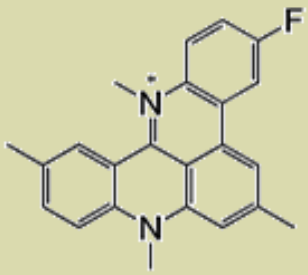
# G4 ligands that reached clinical trials



Telomestatin



R = H, **BSU6039**
R = NH-*p*-$C_6H_4$-$N(CH_3)_2$, **BRACO-19**



**RHPS4**

- Problems with mass production (Monchaud et al. 2010)
- Bioavailability issues (Shin-ya et al. 2010)
- Lack of desirable drug properties (Balasubramanian et al. 2011)

# TMPyP4*

- A porphyrin and a synthetic G4 ligands
- Localise to tumour regions preferentially and to quiescent cancer cells too (Carvalho et al. 1999)
- Bona fide G4 ligand with excellent affinity to quadruplexes (Wheelhouse et al. 1999)
- Poor quadruplex to duplex specificity (in vitro) (De Cian et al. 2005)
- Exquisite quadruplex to duplex specificity (molecular crowding) (Martino et al. 2009)

**mesa-5,10,15,20-Tetrakis-(N-methyl-4-pyridyl)porphine Tatratosylate*

# Telomerase inhibition in brain tumour cells

## Glioblastoma and Medulloblastoma cells

| Cell line | Brain cancer type | Radioresponse |
|---|---|---|
| A172 | Glioblastoma multiforme | Sensitive |
| **ONS76** | **Medulloblastoma** | **Sensitive** |
| **KNS60** | **Glioblastoma multiforme** | **Resistant** |
| U251MG(KO) | Glioblastoma multiforme | Resistant |

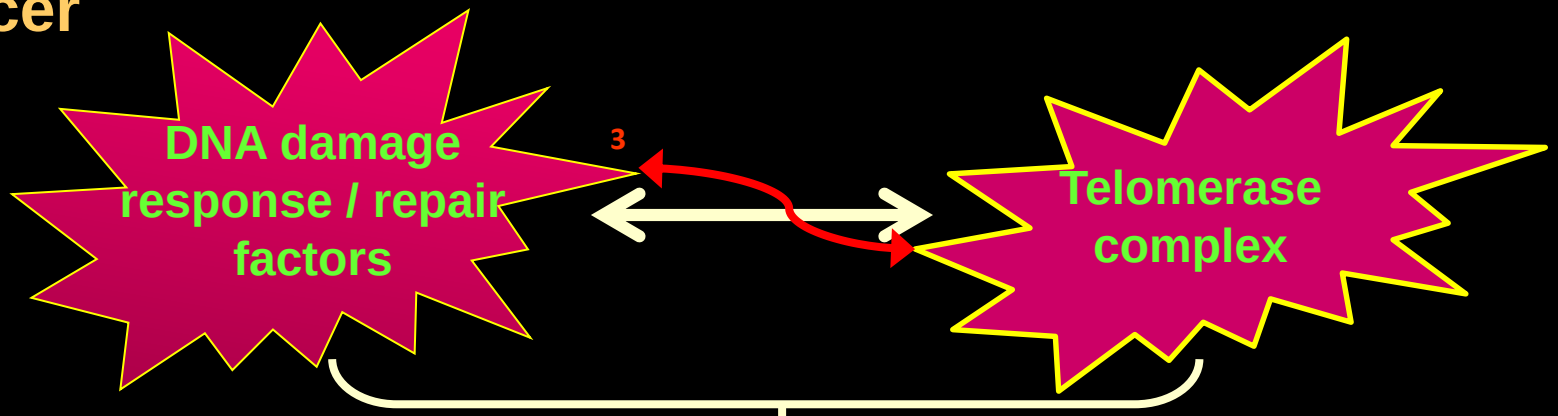
# Results

- TMPyP4 reduces telomerase activity and hTERT levels in human brain cancer cells
- Based on the cytotoxicity assessment after 48 hours, 50 μM of TMPyP4 and 4 Gy of γ-radiation were chosen as the doses for the study.
- Pre-treatment with TMPyP4 potentiates radiation-induced DNA damage
- after 48 hours, 50 μM of TMPyP4 and 4 Gy of γ –radiation were able to reduce the viability of the cancer cells to a modest extent and hence not too cytotoxic.
- TMPyP4 accentuates radiation-induced cell arrest in glioblastoma cells
- TMPyP4 sensitises glioblastoma cells to radiation-induced cell death

TMPyP4 + 4 Gy: Pretreatment with 50 μM TMPyP4 for 24 hours,
then another 24 hours in TMPyP4 after irradiation

**Venkatesan, Sethu and Hande, unpublished**

# Anti-cancer Strategy




Telomere dysfunction

↑sensitivity to mutagens and carcinogens

**Telomere equilibrium**

2 DNA damaging agents

**Telomere length changes**
(end-to-end fusions, translocations, allelic losses, altered gene expression, etc..,)

**Chromosome – Genomic instability**

**Limited proliferative potential / cell cycle arrest**

**Cell Death**
**Senescence ↔ Ageing**

~~Telomerase ↑~~ 1

**Immortalisation / Tumourigenesis**