Hôpital général juif
Jewish General Hospital

McGill

INSTITUT LADY DAVIS DE RECHERCHES MÉDICALES / LADY DAVIS INSTITUTE FOR MEDICAL RESEARCH

Centre Bloomfield de recherche sur le vieillissement

The Bloomfield Centre for Research in Aging

Cancer and Aging: Two Faces of the Same Coin

(3) Telomere Biology and Cancer-Part 2

Hôpital général juif
Jewish General Hospital

McGill

INSTITUT LADY DAVIS DE RECHERCHES MÉDICALES / LADY DAVIS INSTITUTE FOR MEDICAL RESEARCH

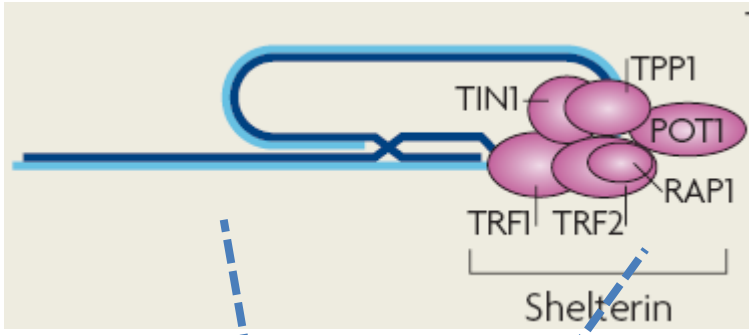
Centre Bloomfield de recherche sur le vieillissement

The Bloomfield Centre for Research in Aging

Targeting Telomerase and Telomeres: Valid Anti-cancer Strategies?

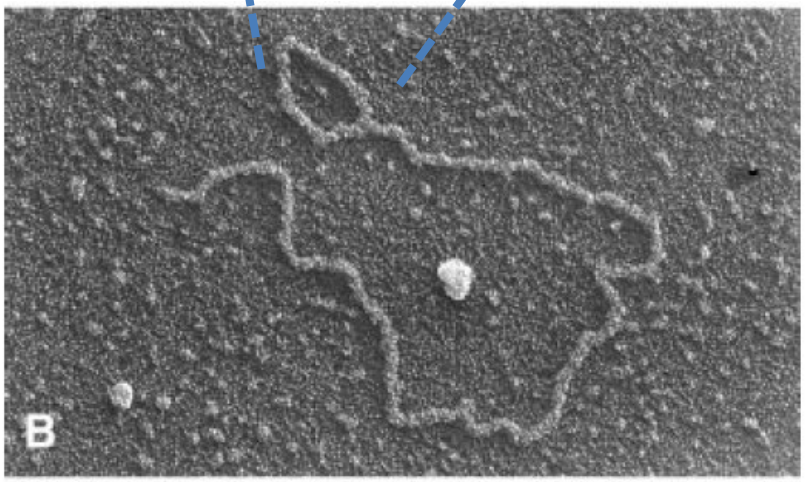
Marie Eve Brault, Johanna Mancini, Hanadi Sleiman, Chantal Autexier

Function of telomeres: cap natural chromosome ends to make them stable structures



End-to-end fusions
Nuclease degradation
Telomere recognized as DNA breaks
Activation of DNA damage response
Senescence/cell death

Murray, JM and Carr, AM
Nat. Rev. Mol. Cell Biol. 2008

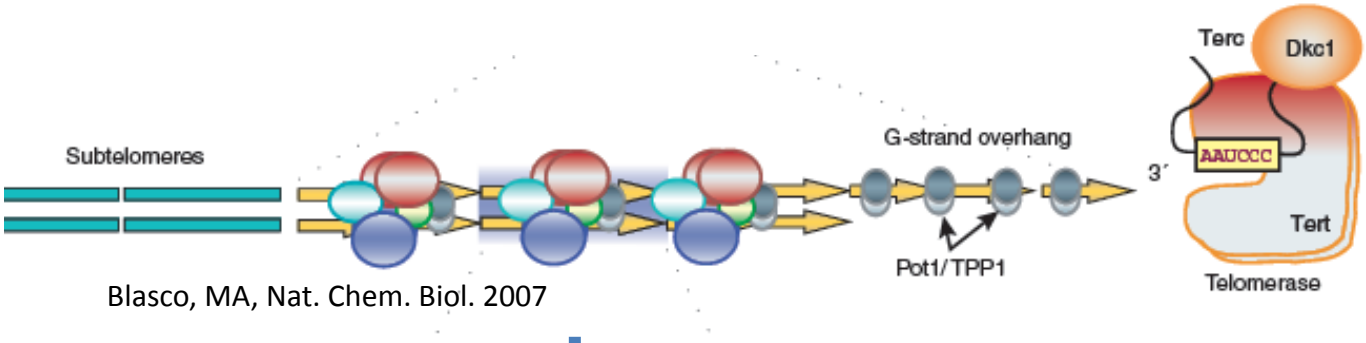


Griffith, JD et al., Cell 1999

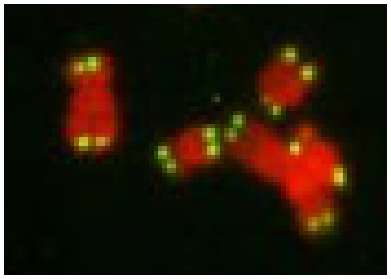
End of chromosome :

Double-strand break:

Function of telomeres: to ensure complete DNA replication at chromosome ends via telomerase

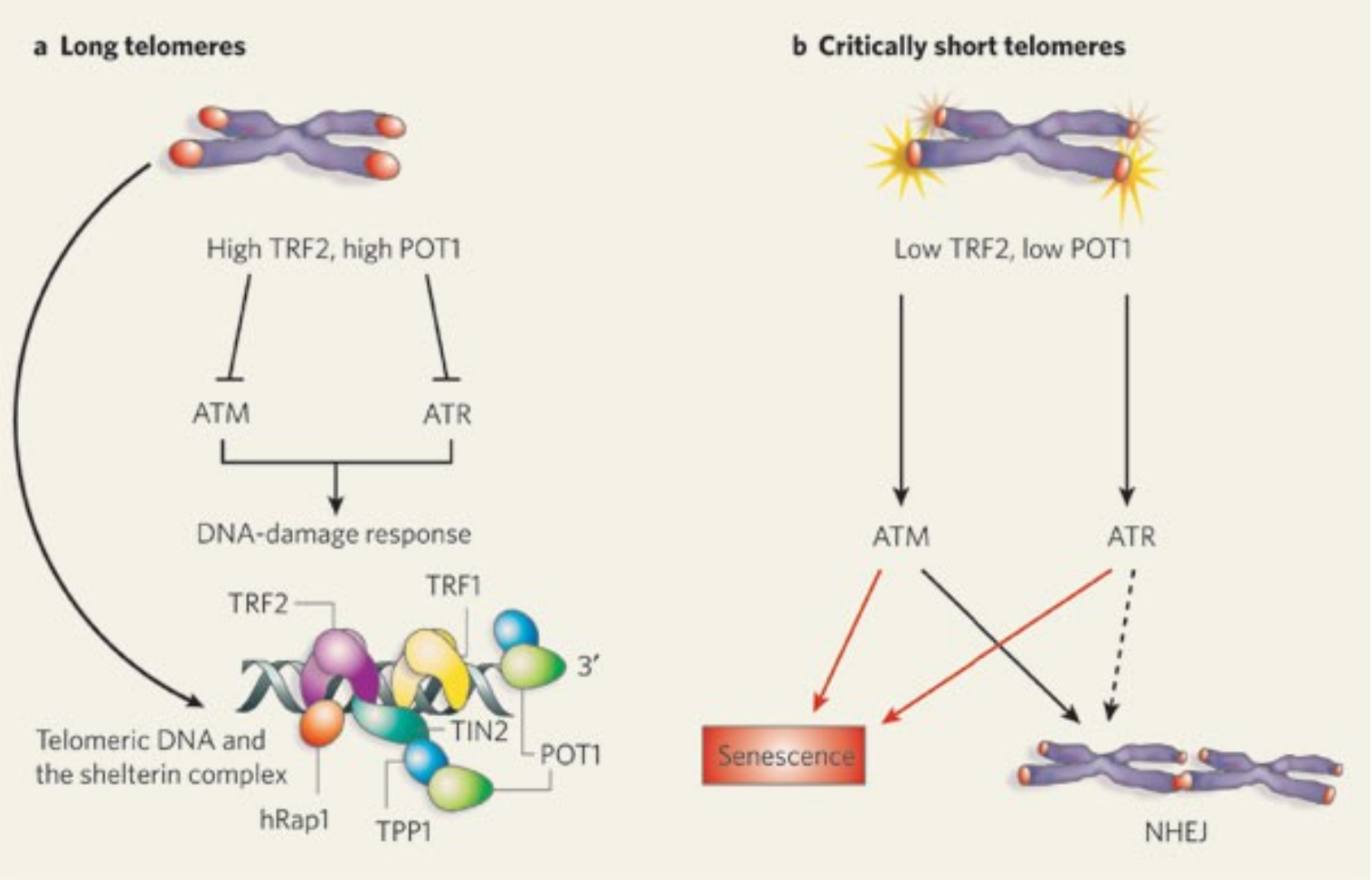


Blasco, MA, Nat. Chem. Biol. 2007



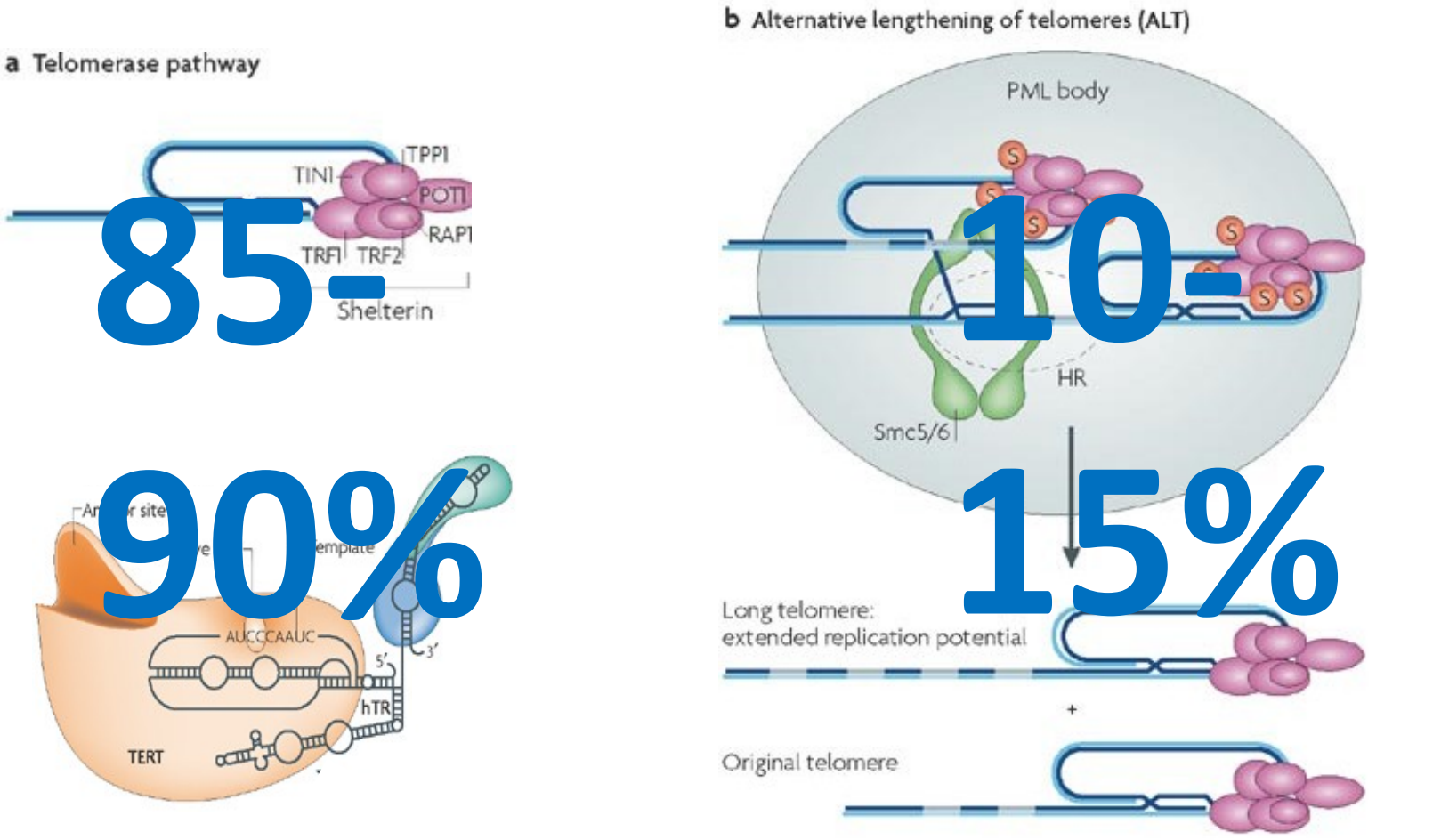
Azzalin et al., 2008

The T-loop structure protects telomeres



CA Azzalin and J Lingner, Nature, 2007

Strategies for telomere length maintenance



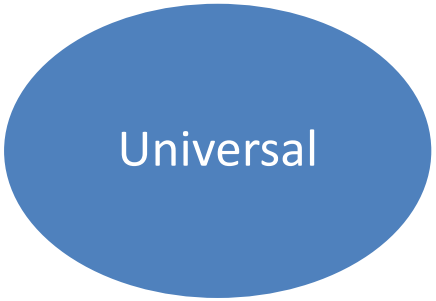
85-90%

10-15%

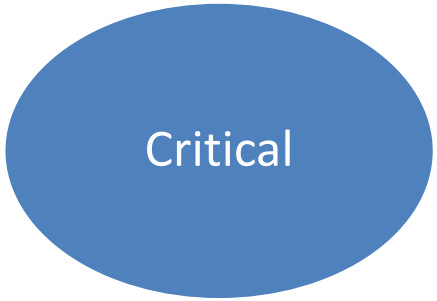
Harley, C.B. Nat. Reviews Canc., 2008

Murray, JM and Carr, AM. Nat. Rev. Mol. Cell Biol. 2008

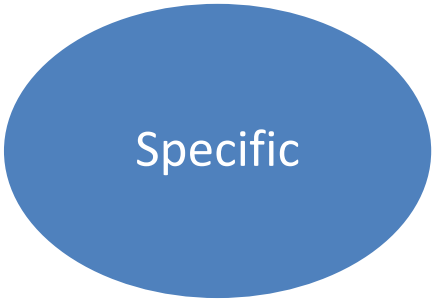
Why does Telomerase Represent a Good Anti-cancer Target?



- 85-90% all tumors are telomerase positive

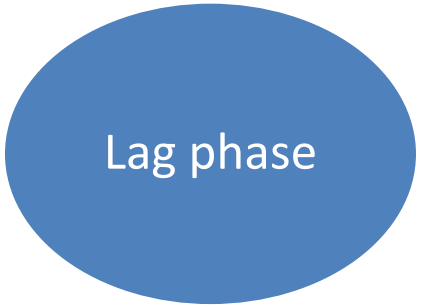


- Telomerase activity/Telomere maintenance is required for the transformed phenotype



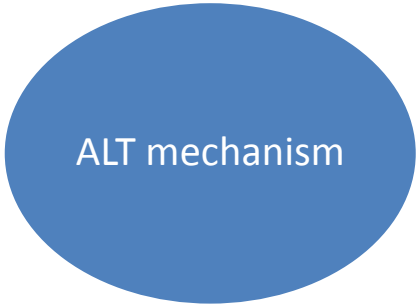
- Most normal human somatic cells have no or very low telomerase whereas cancer cells upregulate telomerase expression

Issues to Consider When Targeting Telomerase Function



- Lag phase between the time telomerase is inhibited and the time telomeres of the cancer cells will have shortened sufficiently to produce detrimental effects on cellular proliferation

- Telomerase inhibitors might result in the emergence of drug-resistant cancer cells (reactivation of telomerase or of the alternative lengthening of telomeres (ALT) pathway)



- Alternative ‘recombination based’ mechanisms for telomere maintenance have been reported in 15% of human cancers.

Telomerase and telomeres as potential targets

Catherine Lauzon

Inhibition of telomere integrity
in combination with chemotherapy

Marie Eve Brault , Nahid Golabi and Johanna Mancini
Regulation of telomere maintenance by recombination

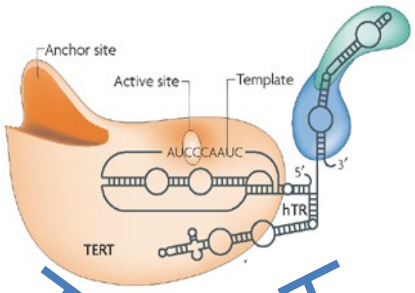
Johanna Mancini and Hanadi Sleiman
Characterization of G-quadruplex ligands as telomerase inhibitors and/or disruptors of telomere integrity in cancer cells

May Shawi and Raquel Aloyz
Inhibition of telomerase in combination with chemotherapy in chronic lymphocytic leukemia

Telomeric recombination as a potential resistance mechanism in response to telomere dysfunction

Targeting the integrity of telomeres versus telomerase

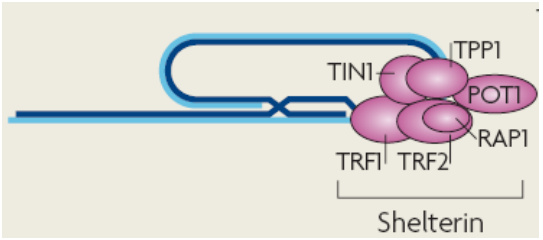
Telomerase



BIBR1532

GRN163L

Telomere



Q-quadruplex ligands
ex: Telomestatin

Telomere erosion
« slow » pathway
Sensescence (↑p16, p21)
Telomere shortening

Telomere uncapping
Rapid pathway
↑ apoptosis
↑ DNA damage response (↑γH2AX, 53BP1, P-ATM)
Displacement of POT1 and/or TRF2
Chromosome fusions
Specific for cancer cells?

Decrease proliferation
Decreased tumor growth in vivo
Enhance the response to cancer drug treatment

Adapted from :
Kelland L. Clin. Cancer. Res., 2007
Harley, C.B. Nat. Reviews Canc., 2008
Murray, JM and Carr, AM
Nat. Rev. Mol. Cell Biol. 2008

Telomere disturbance through the expression of a telomerase RNA with a point mutation in the template region (MuA-hTR)

Telomerase-based anti-cancer approach:

Wild-Type telomerase RNA template 5'-CUAACCCUAA-3' specifies TTAGGG repeats

5' -GGTTAGGGTTAGGGTTAGGGTTAGGGTTAG GGTTAGGGTTAGGGTTAG
3' -CCAAT

Mutant telomerase RNA template 5'-CAAACCCAAA-3' specifies TTTGGG repeats

5' -GGTTAGGGTTAGGGTTAGGGTTAGGGTTAG GGTTTGGGTTTGGGTTTG
3' -CCAAT

-Tumor specific
(telomerase-dependent)

- No lag phase
- General

Anticancer strategies targeting telomere function by expression of template-mutated hTR

- Growth inhibition
- Altered cell cycle
- Apoptosis
- Senescence
- No effect on telomerase-negative lung fibroblasts

- Loss of tumor growth in xenografts

- Dependent on assembly of an active enzyme
- DNA damage response at telomeres
- Chromosome fusions
- Anaphase bridges

- Independent of p53 status
- Can be ATM dependent

Marusic L. et al. (1997) *Mol. Cell. Biol.* 17: 6394-6401; Guiducci, C. et al. (2001) *Oncogene* 20: 714-725; Kim MM. et al. (2001) *Proc. Natl. Acad. Sci.* 98: 7982-7987; Li S. et al. (2004) *Cancer Res*. 64:4833-4840; Goldkorn, A. and Blackburn, E.H. (2006*) Cancer Res.* 66: 5763-5771; Cerone, MA et al. (2006) Oncogene 25: 7411-7420; Stohr, B.A. and Blackburn, E.H. (2008)Cancer Res. 68: 5309-5317; Mahalingam, D. et al. (2011) FEBS J. 278, 3724-3738.

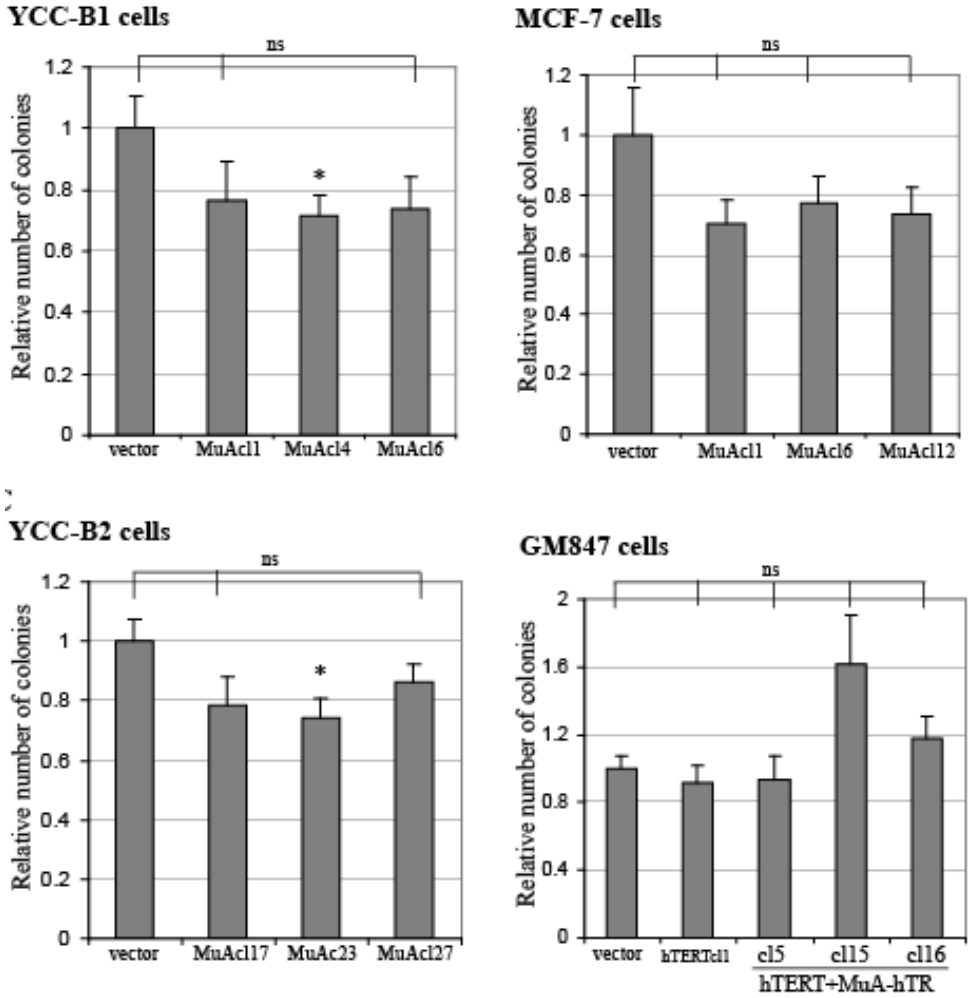
Generation of cancer cell lines with various telomere lengths expressing template-mutated hTR

	YCC-B1	YCC-B2	MCF-7	GM-847
Telomere length (kb)	3.2 (short)	11 (long)	7 (intermediate)	>20 (ALT)
Telomerase	+	+	+	-

Determine effects on cell viability and proliferation upon treatment with chemotherapeutic drugs

Cerone, M.A., Londoño-Vallejo, J.A. and Autexier, C. 2006 Mutated telomeres sensitize tumor cells to anticancer drugs independently of telomere shortening and mechanisms of telomere maintenance. Oncogene 25, 2411-2420.

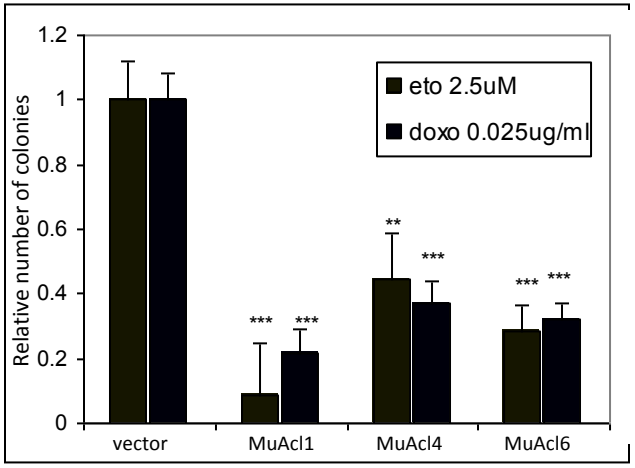
MuA-hTR has a mild effect on cell viability and proliferative ability



Cerone, M.A., Londoño-Vallejo, J.A. and Autexier, C. Oncogene, 2006.

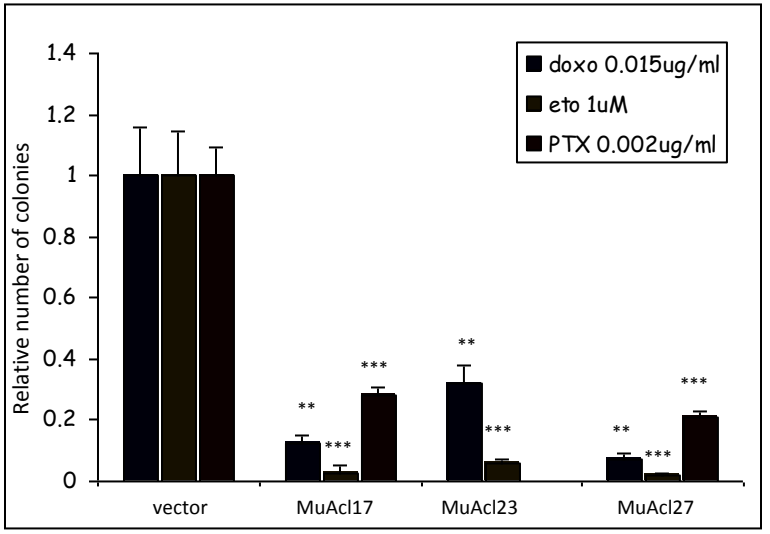
MuA-hTR expression increases the sensitivity of cancer cells to chemotherapeutic drugs independently of telomere length and initial telomerase status

YCC-B1 (short TRF)



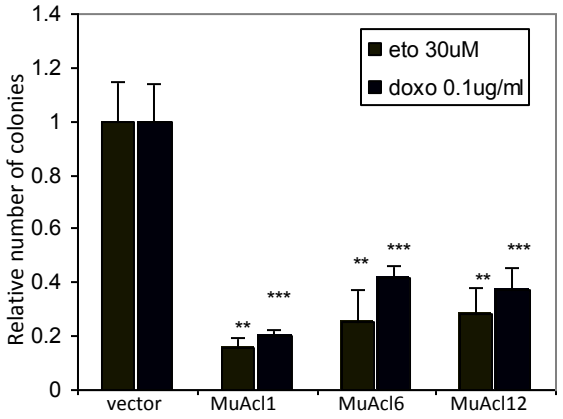
**p<0.01; ***p<0.0001

YCC-B2 (long TRF)



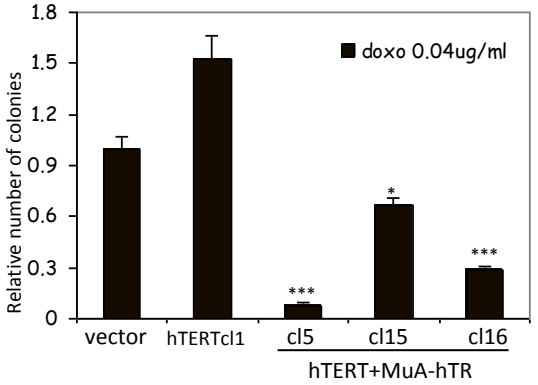
**p<0.01; ***p<0.0001

MCF-7 (intermediate TRF)



**p<0.01; ***p<0.0001

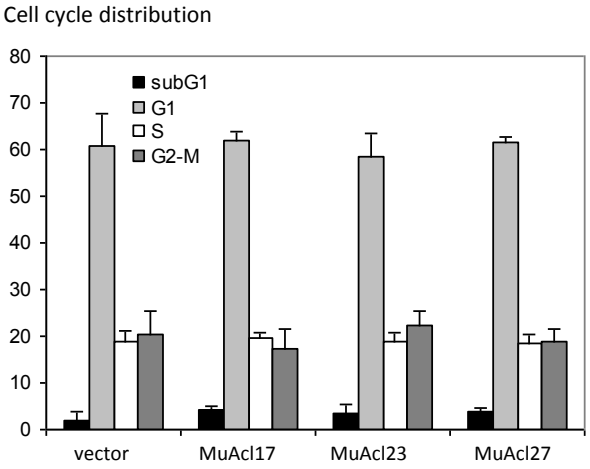
GM847 (ALT)

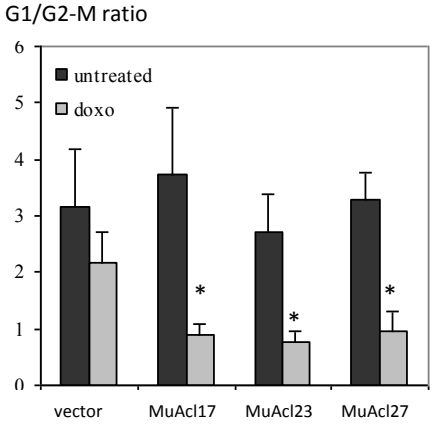


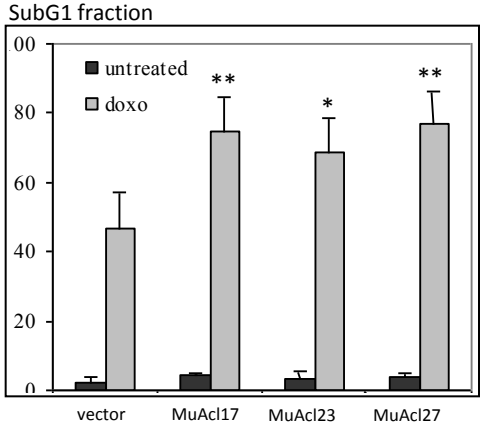
*p<0.05; ***p<0.001

Cerone, M.A., Londoño-Vallejo, J.A. and Autexier, C. Oncogene, 2006.

MuA-hTR expression alters the cell cycle profile of YCC-B2 cells after drug treatment perhaps leading to the increased sensitization to drugs

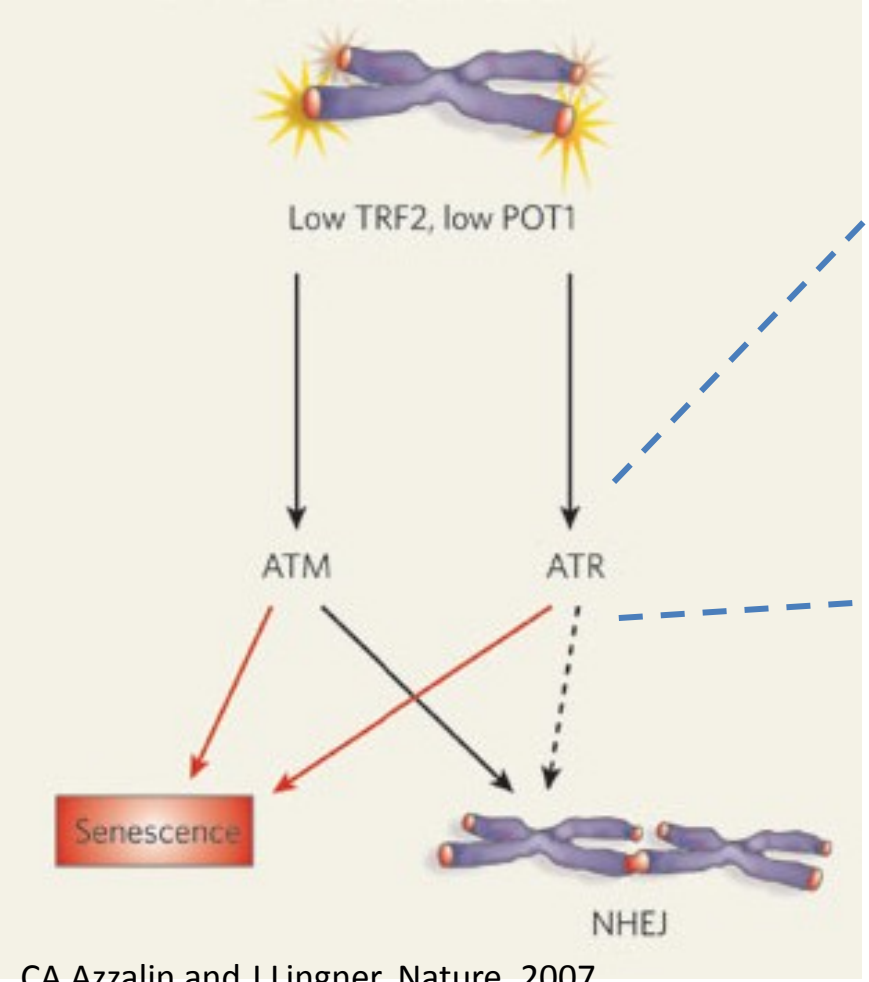






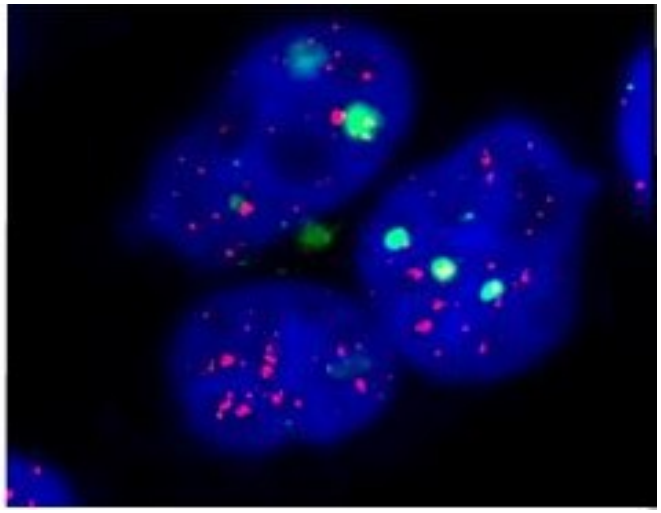
Cerone, M.A., Londoño-Vallejo, J.A. and Autexier, C. Oncogene, 2006.

DNA damage response at the telomeres : Telomere Dysfunction-Induced Foci (TIFs)



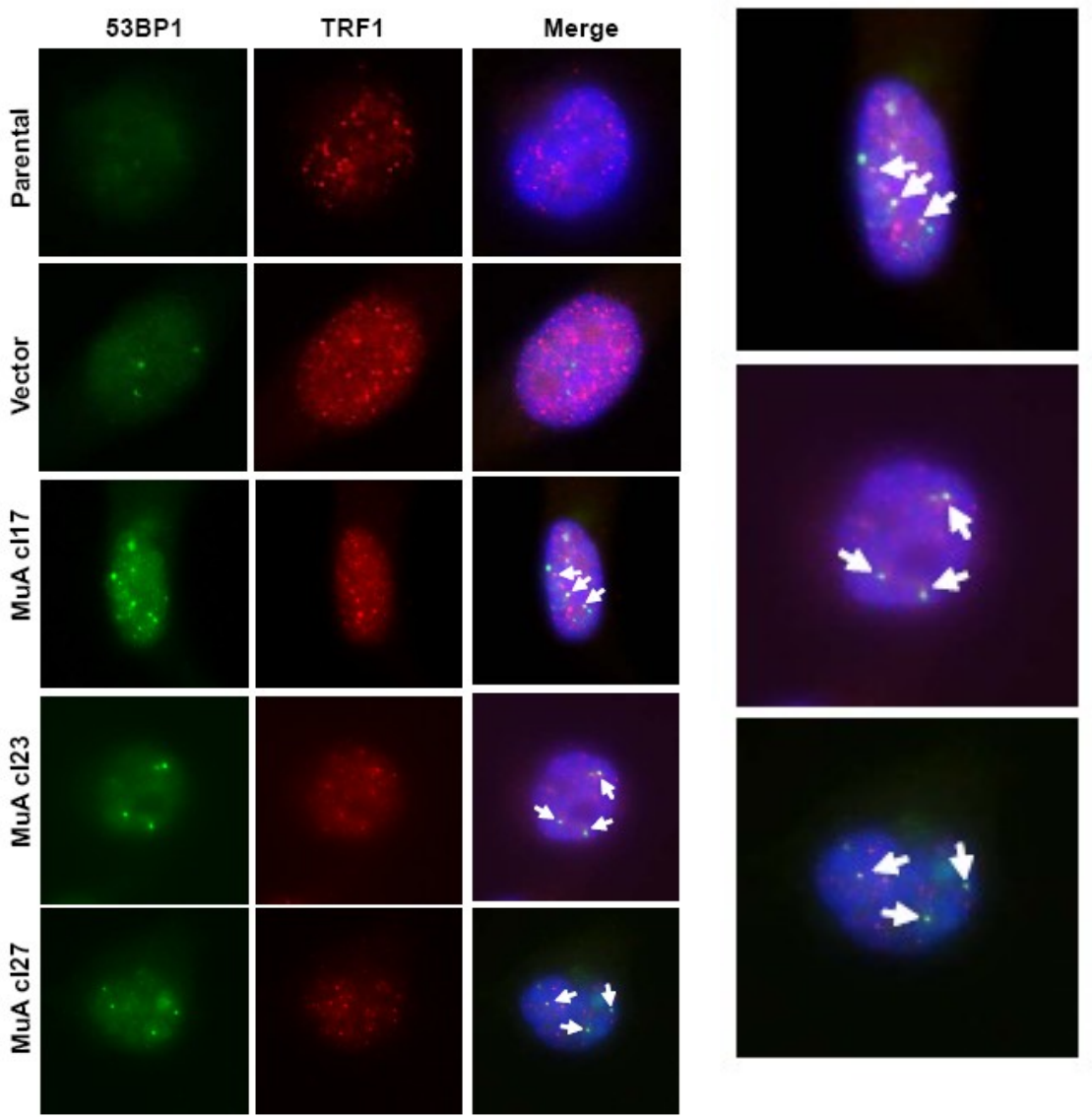
CA Azzalin and J Lingner, Nature, 2007

Telomere dysfunction induced-foci (TIFs)



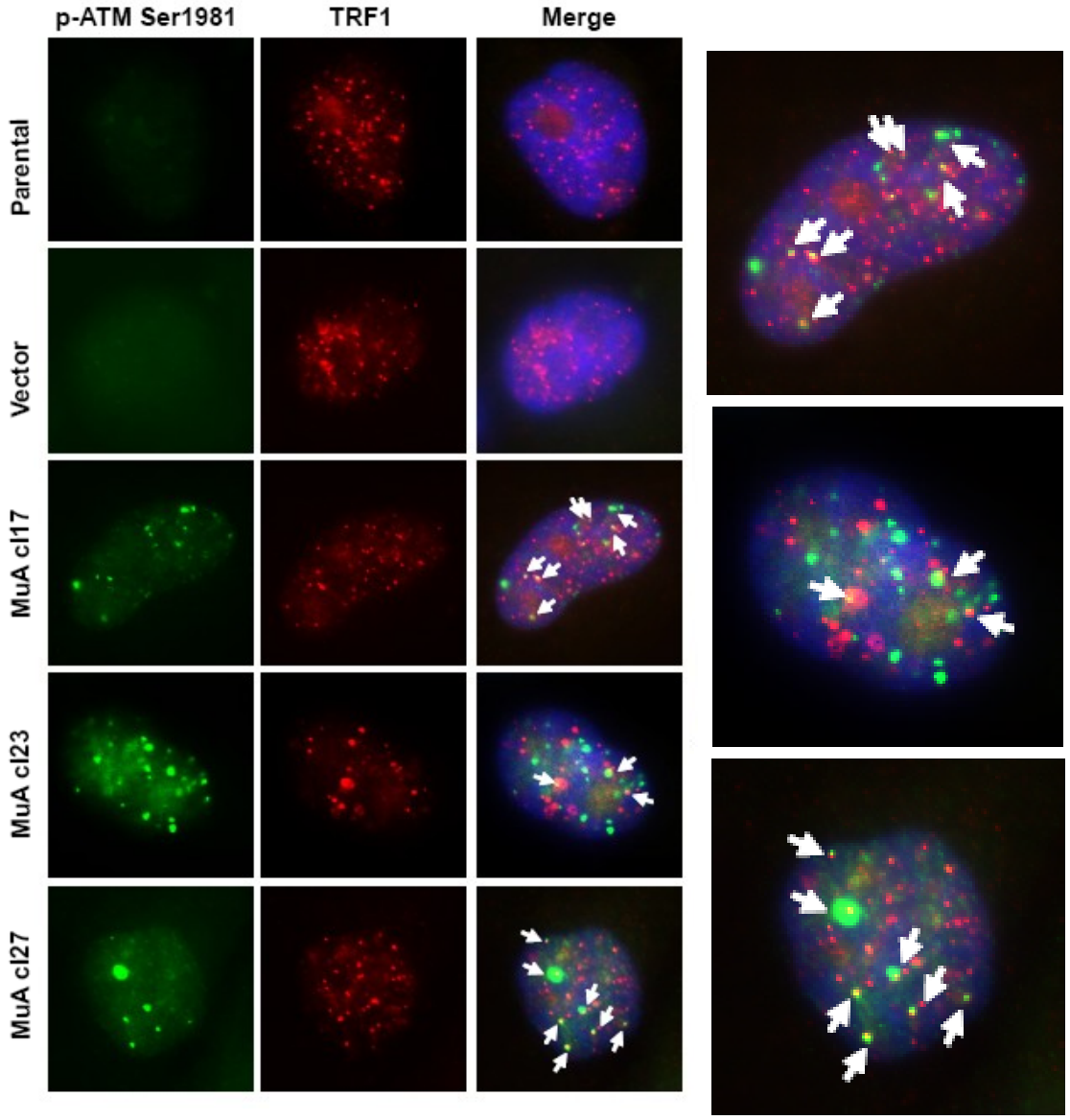
53BP1
γH2AX
p-ATM
MRN
MDC1

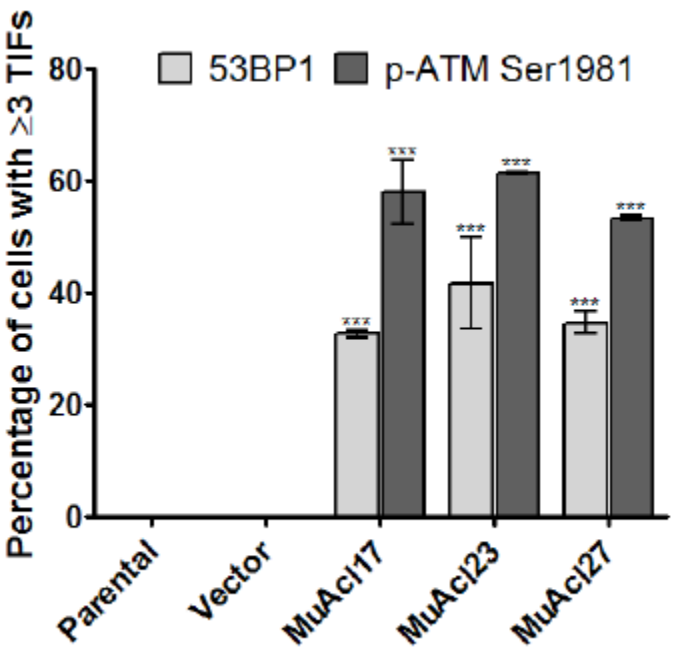
MuA-hTR induces the formation of TIFs containing TRF1 and 53BP1 in YCC-B2 cells



Brault and Autexier, MBC, 2011

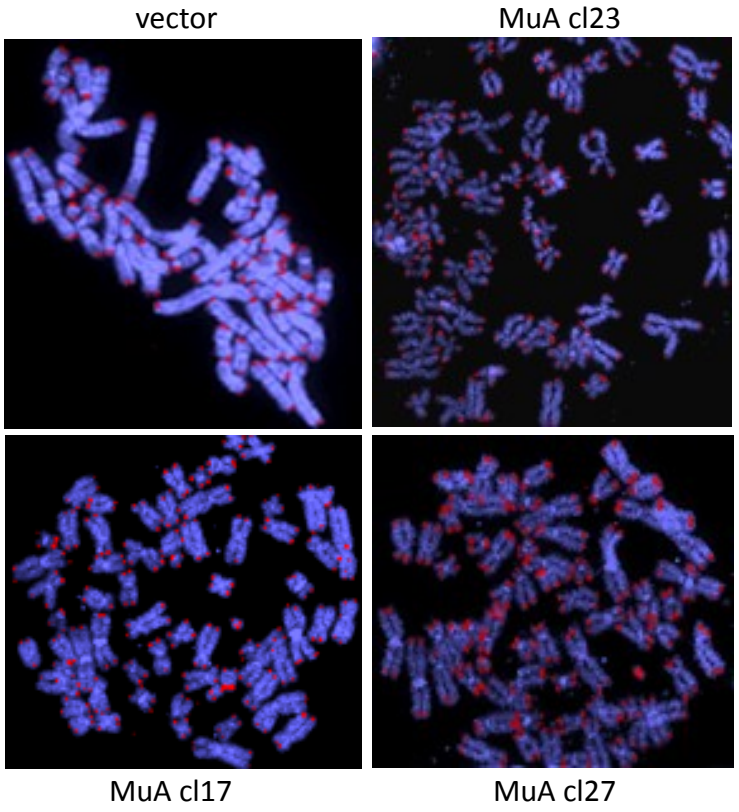
MuA-hTR induces the formation of TIFs containing TRF1 and P-ATM Ser 1981 in YCC-B2 cells

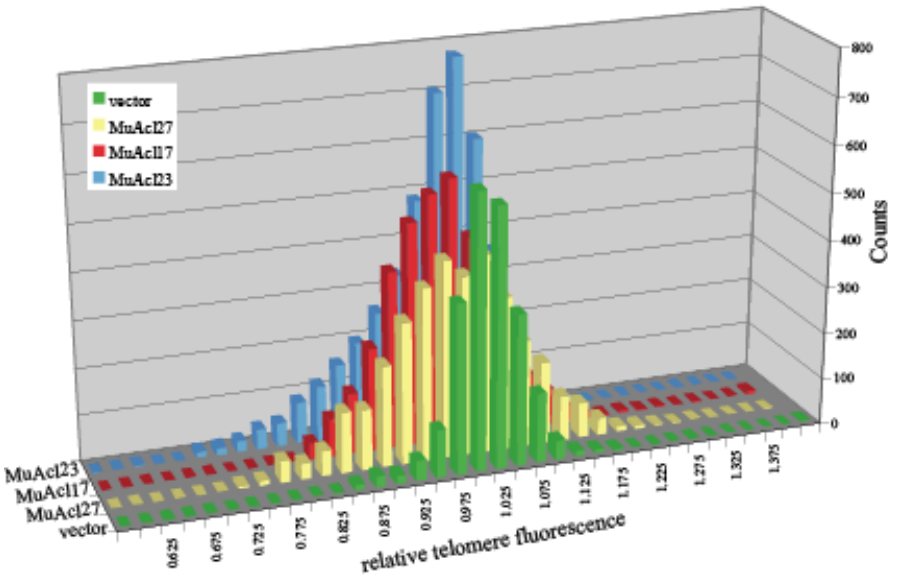




Brault and Autexier, MBC, 2011

Observed a broader distribution of relative telomere lengths within cells carrying the mutant RNA, suggesting possible telomeric recombination events





Cerone, M.A., Londoño-Vallejo, J.A. and Autexier, C. Oncogene, 2006.

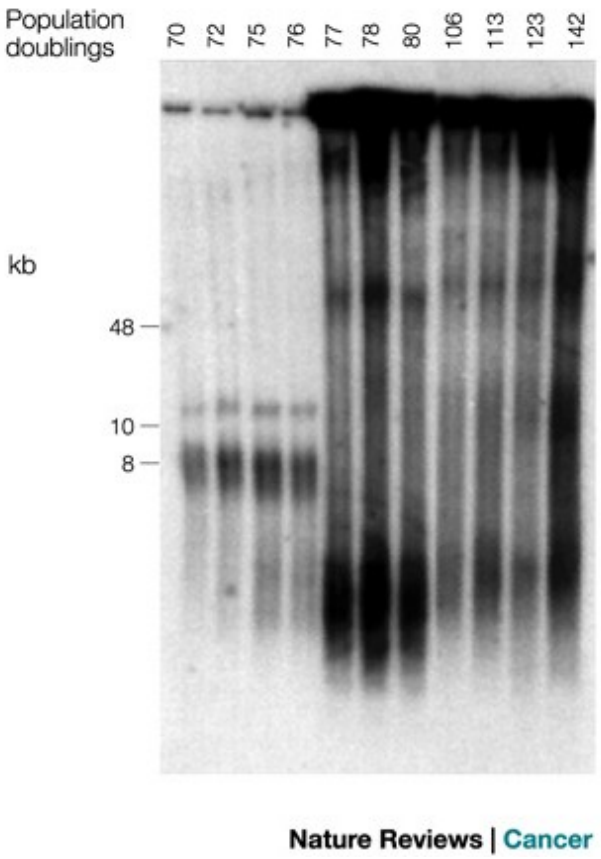
Characteristics of ALT cells

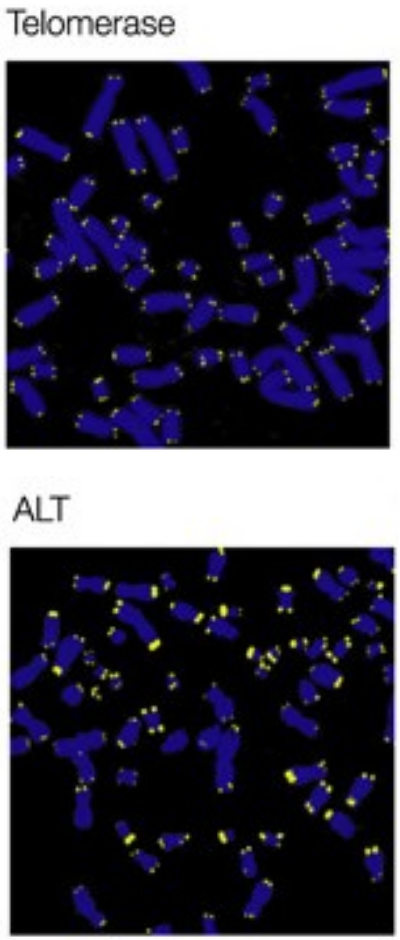
Homologous recombination (HR) mediated events leading to:

1. exceptionally long and heterogeneous telomeres, ranging from <2kb to >50kb
2. High levels of telomeric sister chromatid exchange (T-SCE)
3. Altered pq-ratios
4. extrachromosomal telomeric DNA, of circular (termed t-circles) forms

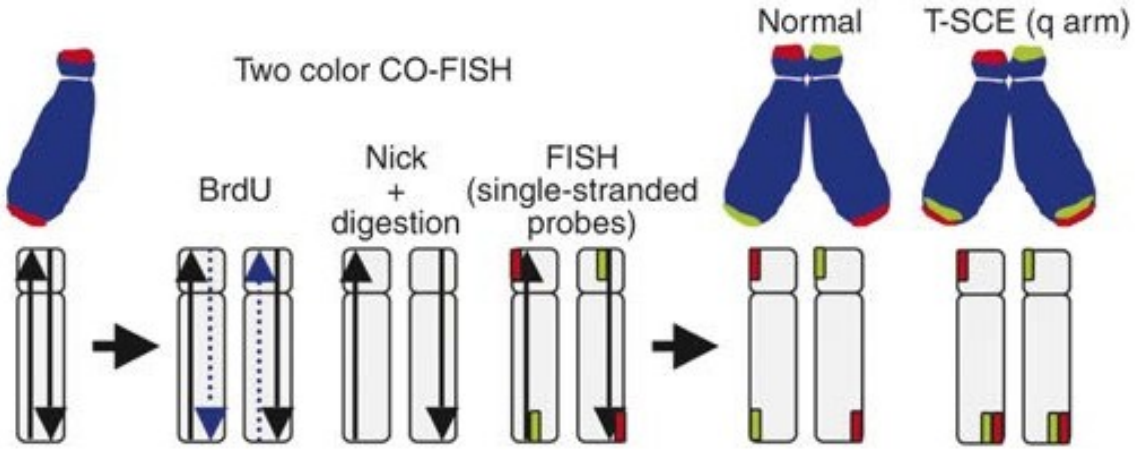
ALT-associated promyelocytic leukemia bodies (APBs) which may be the sites of recombination

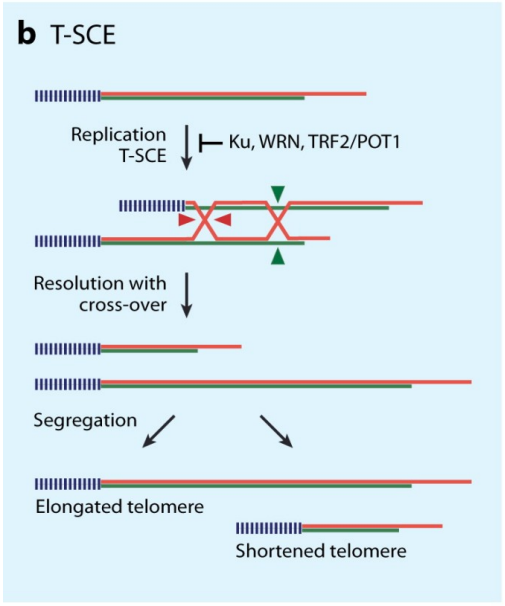
The telomeres in ALT cells are highly heterogeneous and extremely long



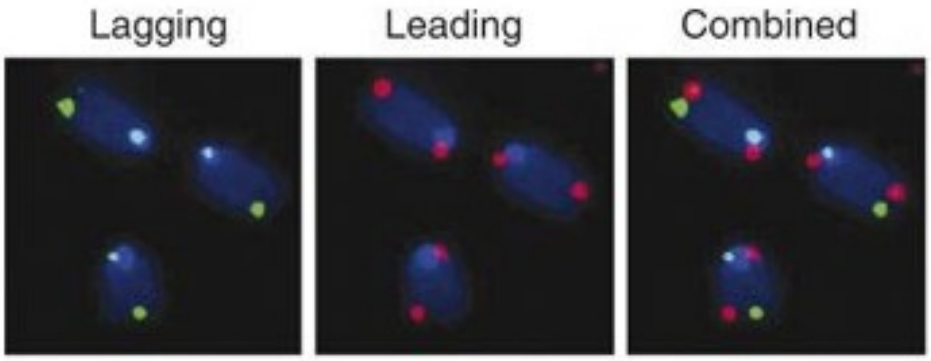


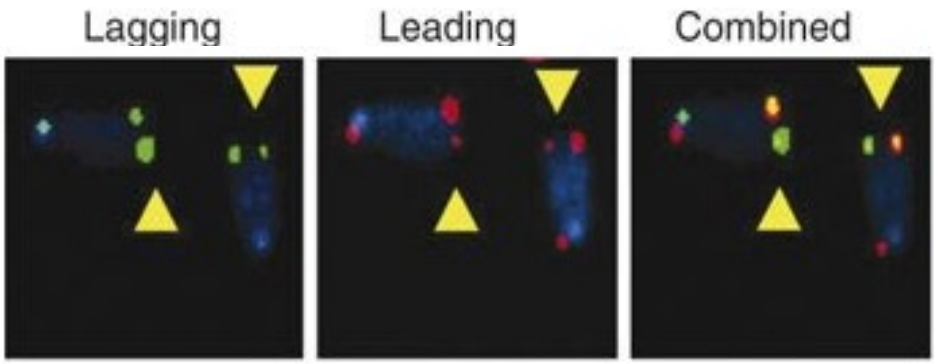
Measuring Telomere-Sister Chromatid Exchanges (T-SCEs) : the CO-FISH Technique



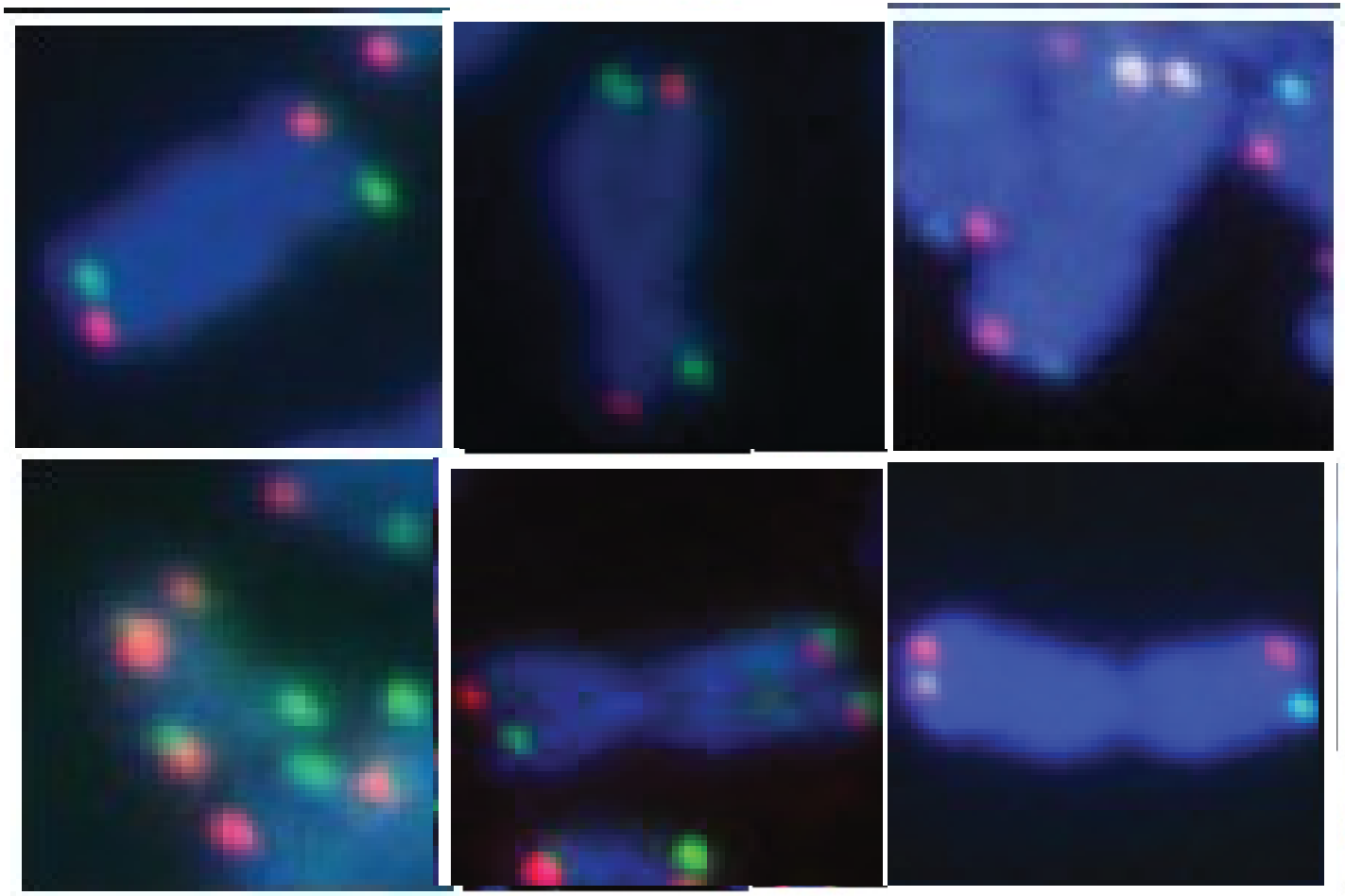


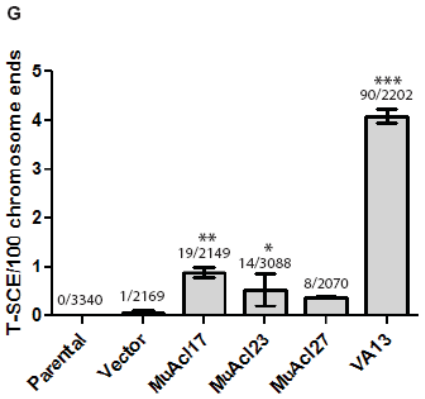
Palm, W. and de Lange, T. Annu. Rev. Genet. 2008.





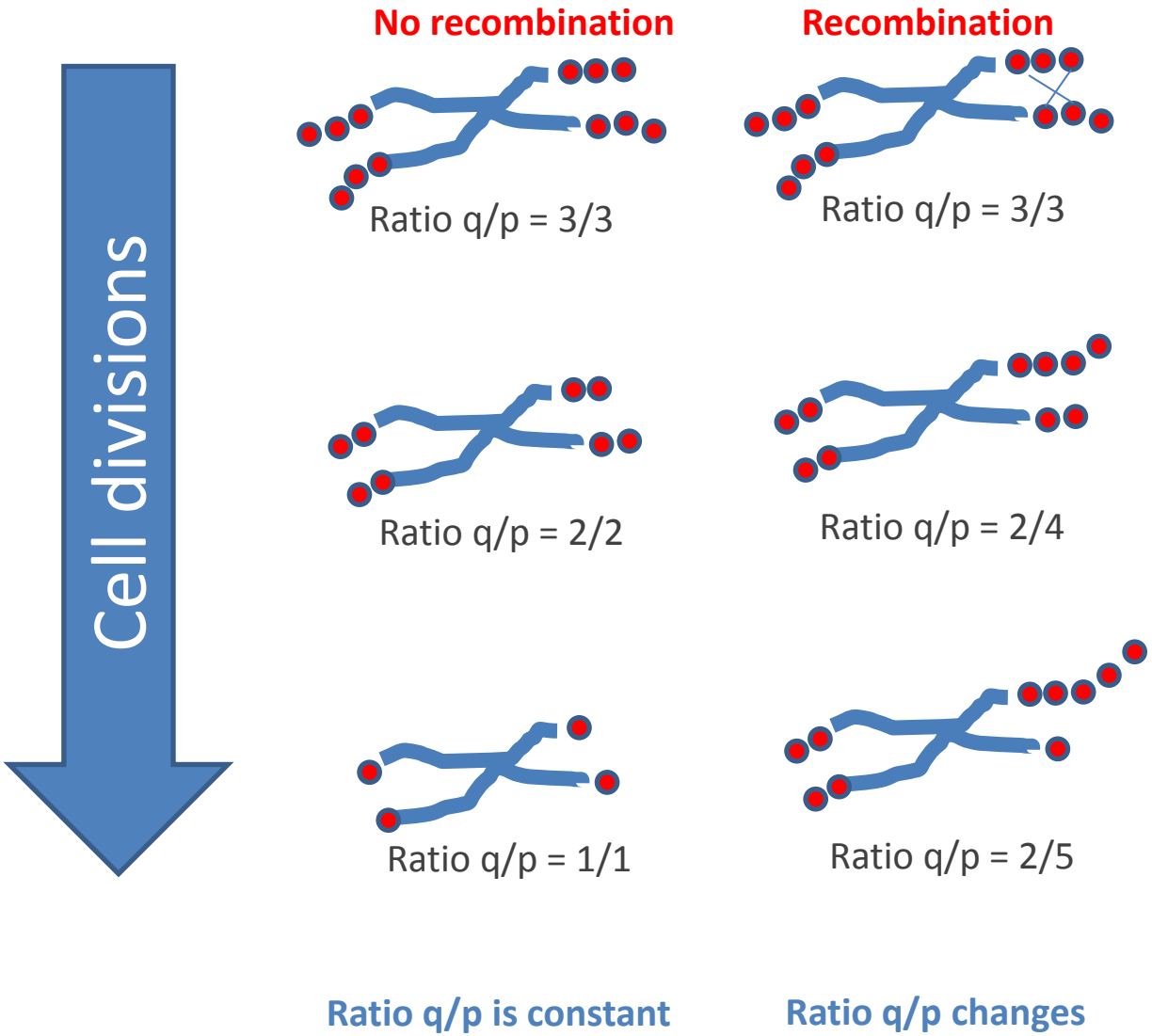
Increased frequency of T-SCEs in the YCC-B2 cells after MuA-hTR expression



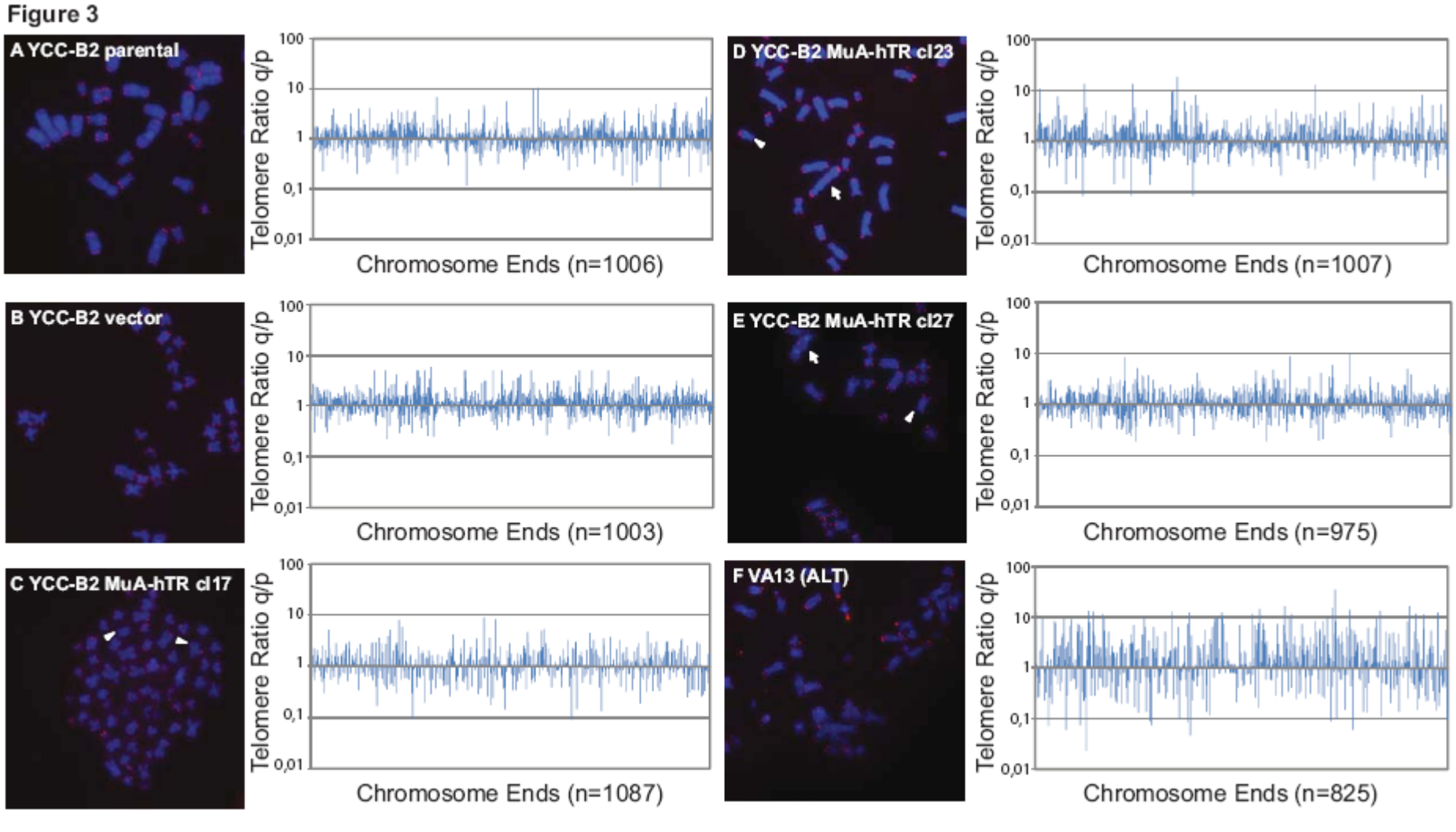


Brault and Autexier, MBC, 2011

Changes in pq-ratios indicate telomeric recombination

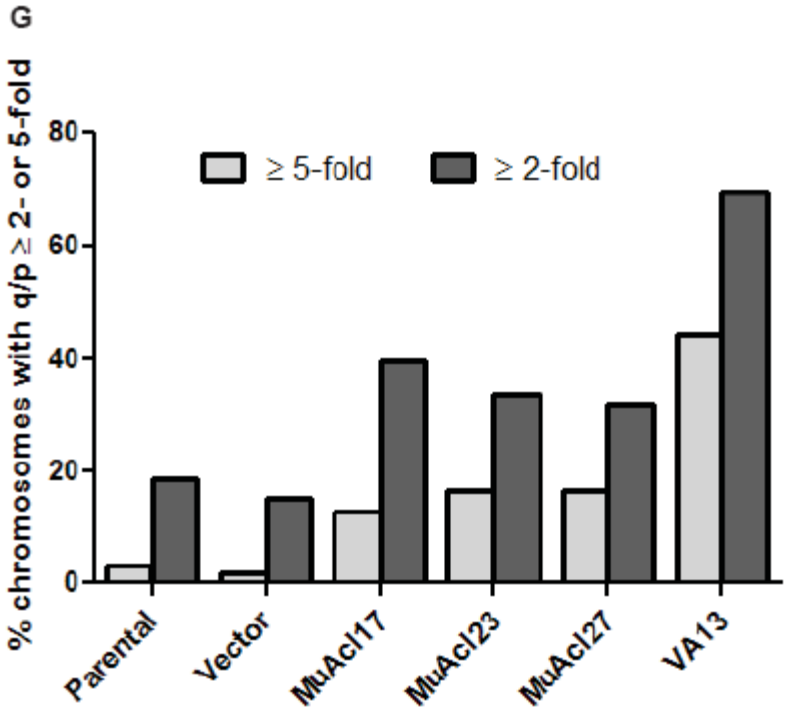


Mutant telomerase RNA expression is associated with changes in telomere pq-ratios



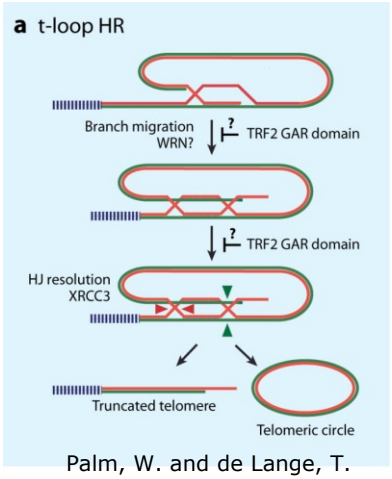
Brault and Autexier, MBC, 2011

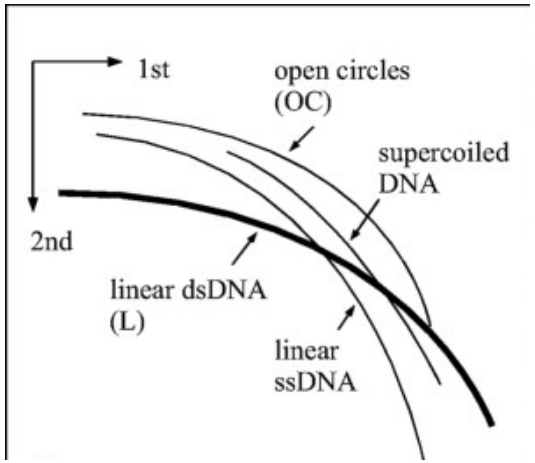
Mutant telomerase RNA expression is associated with changes in telomere pq-ratios



Brault and Autexier, MBC, 2011

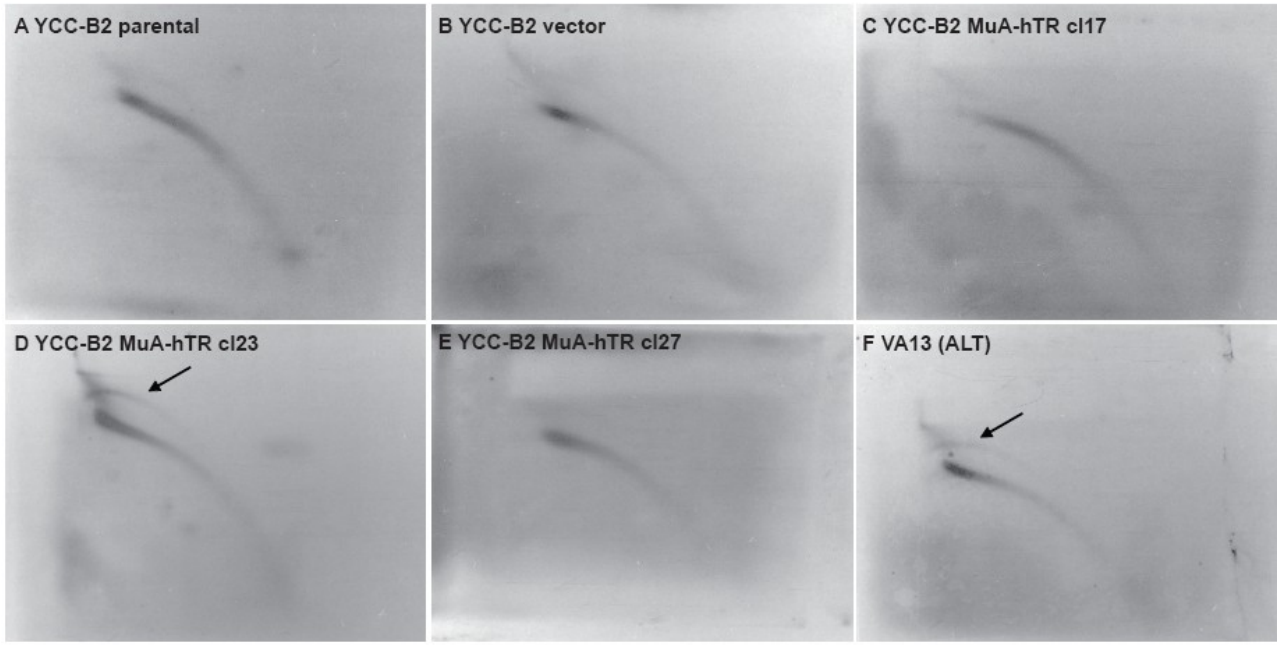
MuA-hTR expression results in the accumulation of circular extrachromosomal telomeric DNA





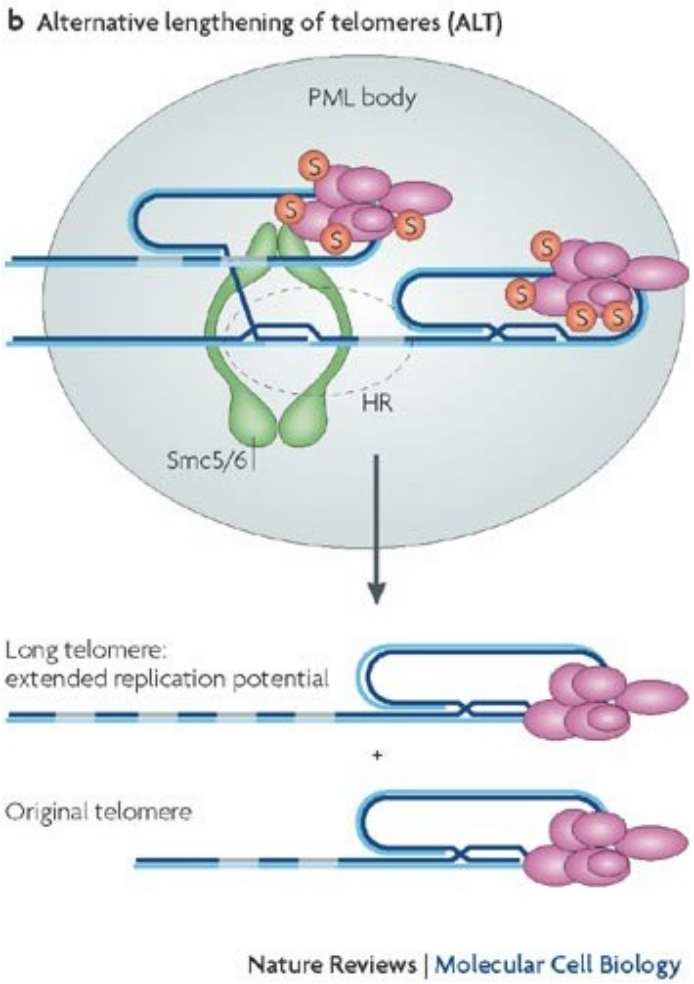
Palm, W. and de Lange, T.
Annu. Rev. Genet. 2008.

Figure 4

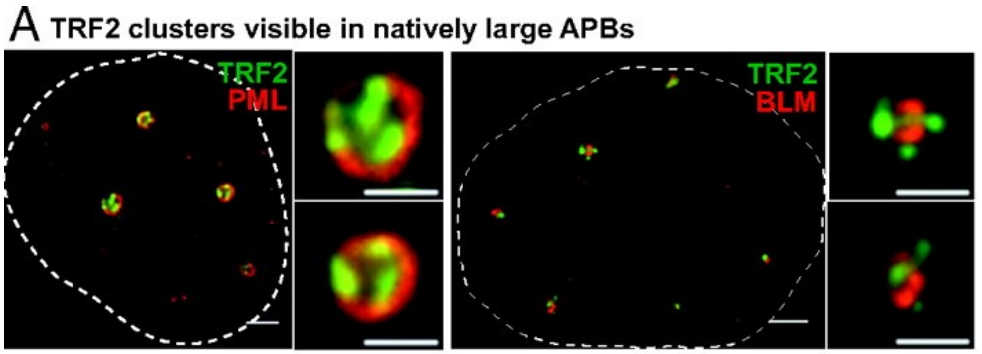


Brault and Autexier, MBC, 2011

ALT cells contain a high level of ALT associated promyelocytic leukemia bodies (APBs)

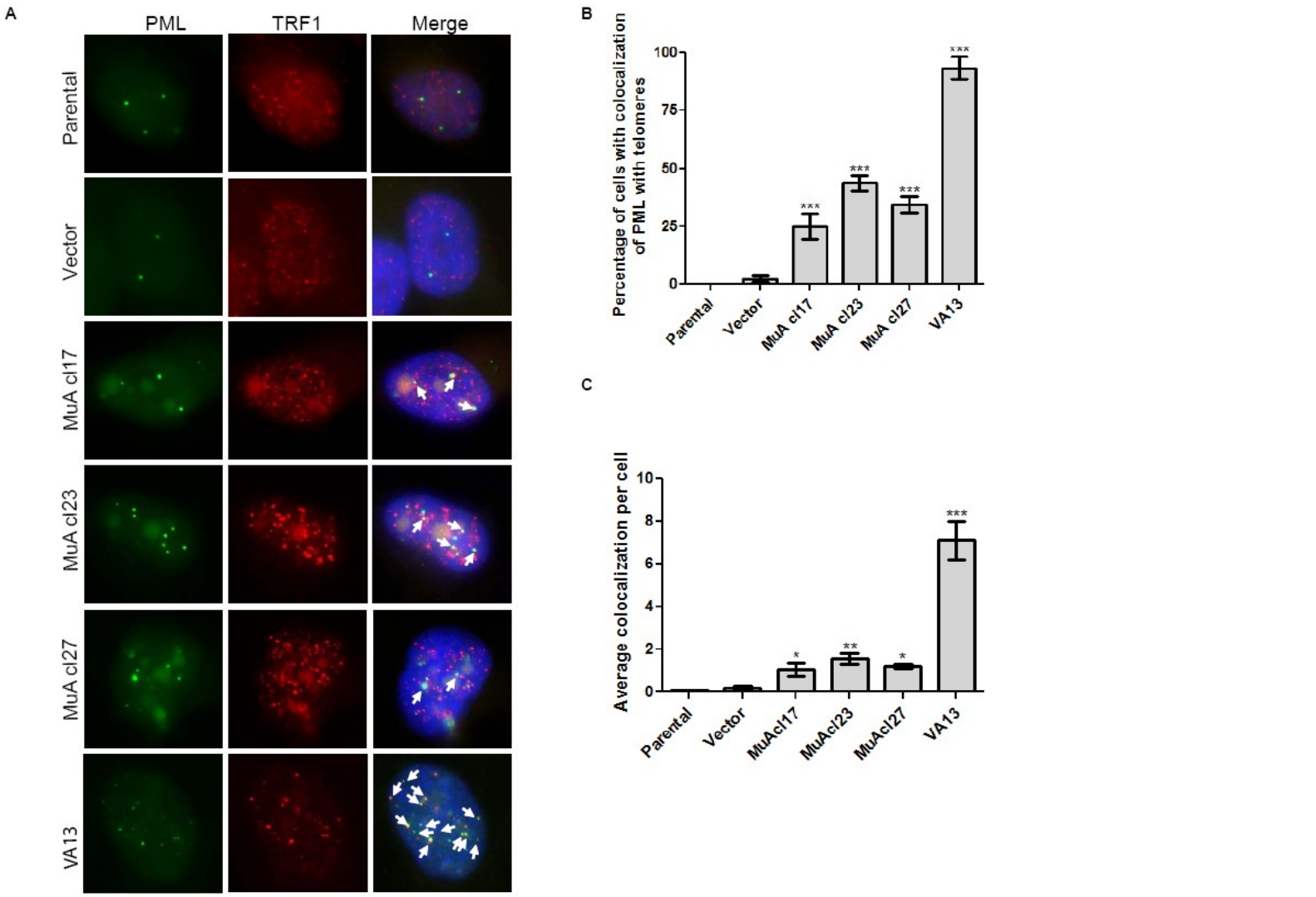


Murray, JM and Carr, AM. Nat. Rev. Mol. Cell Biol. 2008



Irena Draskovic et al., PNAS, 2009.

Increased formation of PML bodies associated with telomeric DNA in telomerase-positive cells expressing mutant telomerase RNA



Brault and Autexier, MBC, 2011

Telomeric recombination induced by dysfunctional telomeres

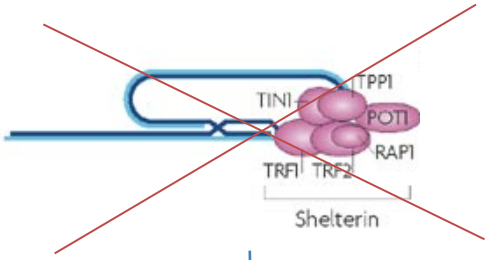
- Elevated DNA damage response located at the telomeres (Telomere dysfunction-induced foci), suggesting that the incorporation of mutant repeats disturbs the telomere cap
- Increased frequency of Telomere Sister Chromatid Exchange (T-SCEs), elevated pq-ratios and extrachromosomal telomeric DNA fragments were observed in the MuA-expressing cells, which are consequences of homologous recombination between telomeres
- First report of telomere dysfunction inducing telomeric recombination in mammalian cells without telomerase inhibition and of endogenous telomerase and telomeric recombination pathways coexisting spontaneously in cancer cells

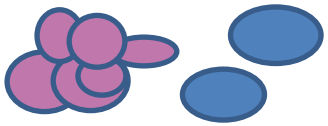
Conclusion

Our results suggest that after telomere destabilization, there is a strong selection pressure for the emergence of resistant cells with increased telomeric recombination and telomeric recombination could be a potential resistance mechanism in response to telomere dysfunction

However, the use of chemotherapeutic drugs decreases the proliferation of the MuA-expressing cells, despite the presence of a recombination-based mechanism for telomere maintenance

Model

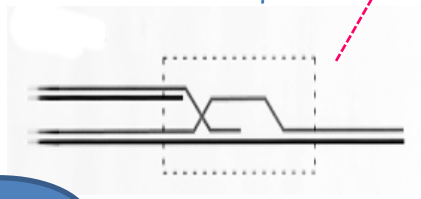




TRF1, TRF2 and POT1 cannot bind to mutant sequences

Telomere maintenance independent of telomerase and survival

↓proliferation
↑apoptosis



MRN

Rad52

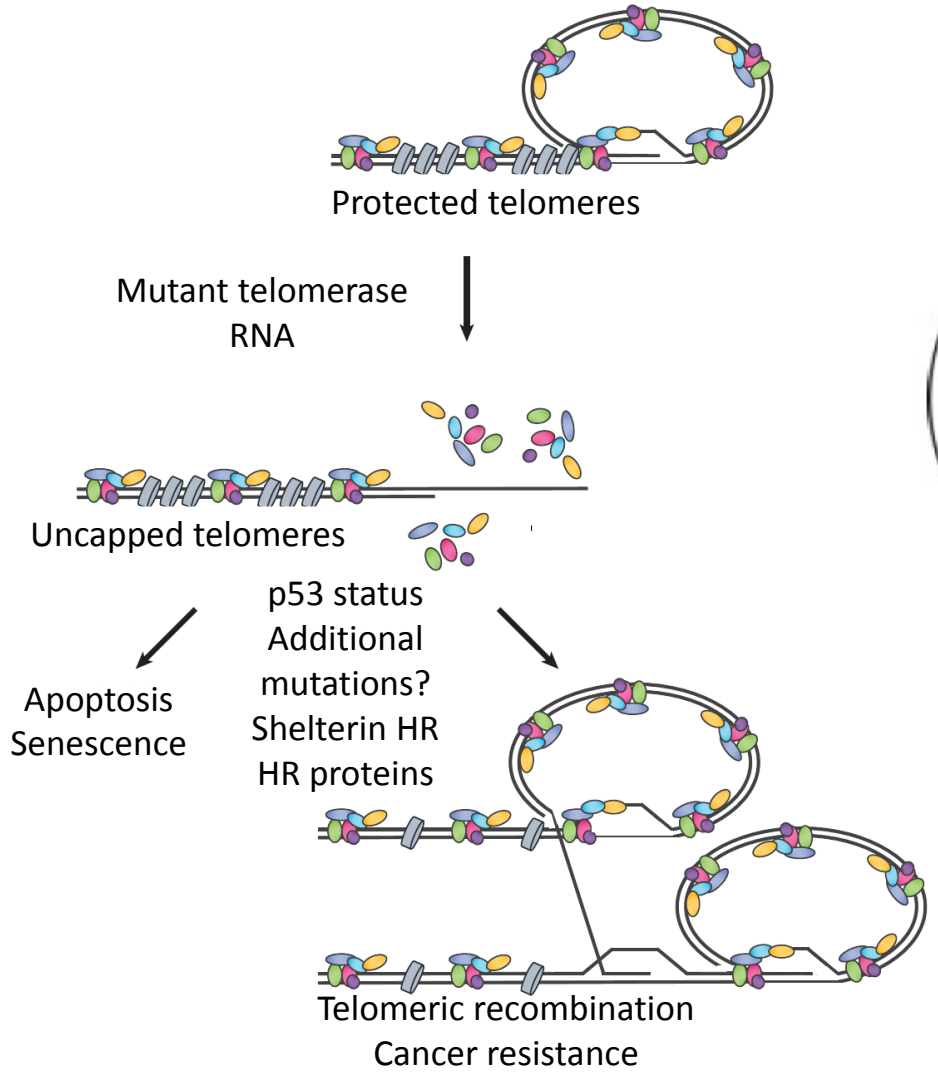
HR proteins favors telomere recombination rather than T-loop formation

↑instability

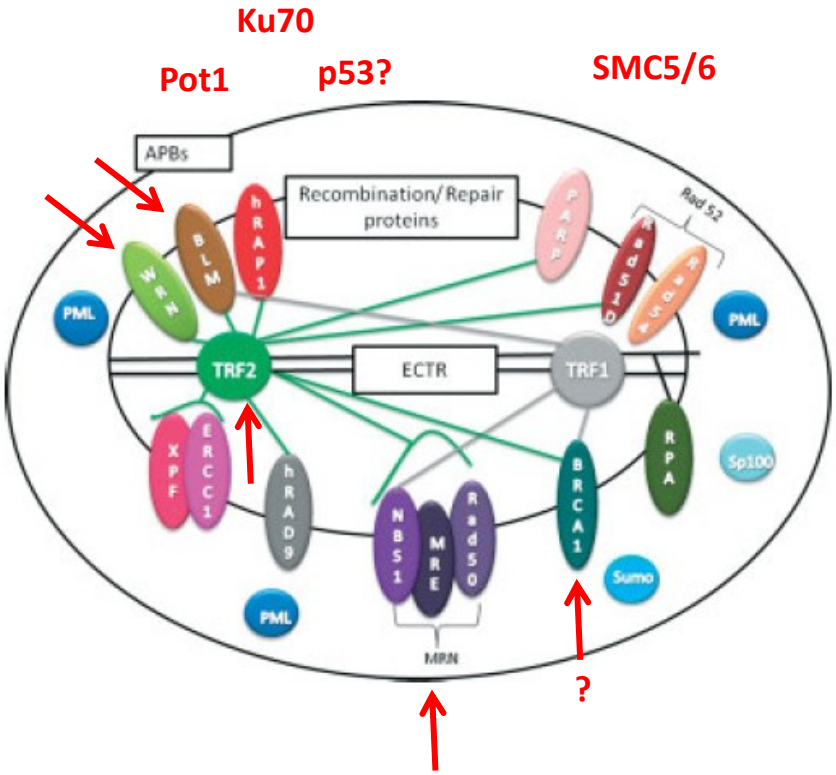
Drugs

Perspective

The factors involved in resistance to induced-telomere dysfunction could include the impaired p53 status of YCC-B2 and additional proteins which regulate or may regulate ALT or telomeric recombination



Adapted from Brault and Autexier, Cell Cycle, 2011



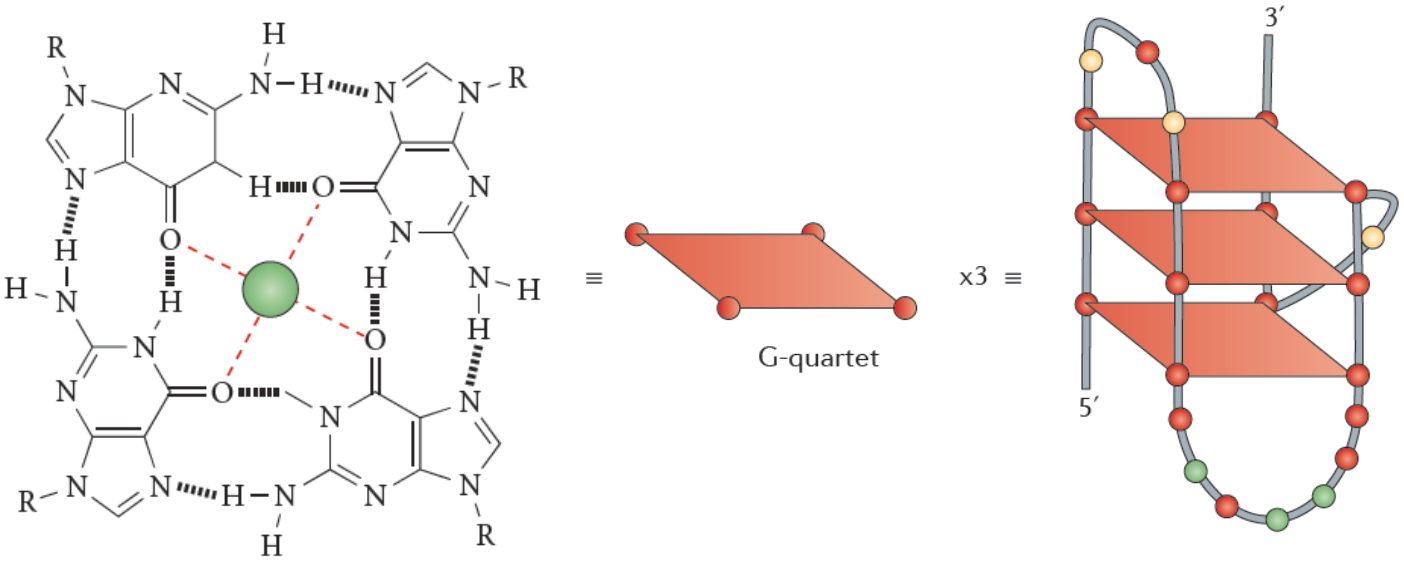
Adapted from De Boeck, G. et al. J. of Path. 2008.

Inhibiting telomerase and telomere function in cancer cells with G-quadruplex ligands

G-quartet

- Bang 1910
- Gellert *et al* 1962
- 4 Guanines

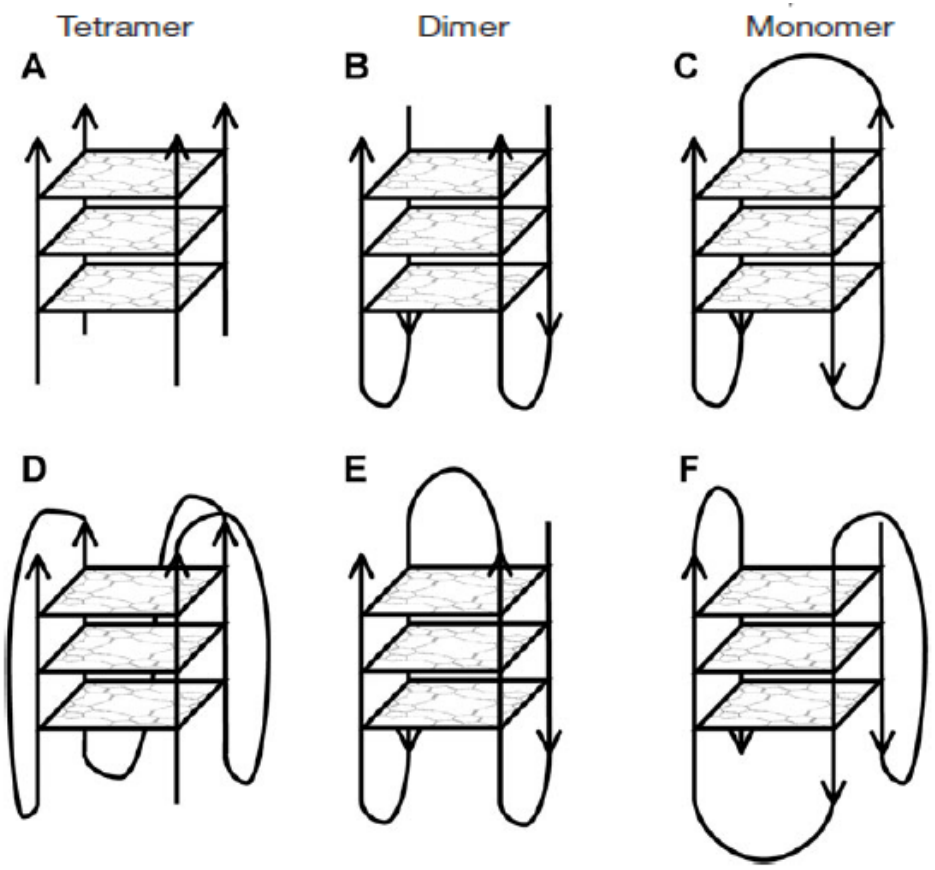
- Stabilized by Hoogsteen Hydrogen bonds
- Stacked = G-quadruplex
- Stabilized by K^+ and Na^+



Balasubramanian *et al* *Nature Reviews Drug Discovery* (2011)

G-quadruplex

- Forms determined by:
  - Strand number
    - Monomer, Dimer, Tetramer
  - Strand direction
    - Parallel, anti-parallel
  - Loops
    - Lateral/Edgewise
    - Diagonal
    - Chain-reversal/Propeller
  - D, E, & F formed in human telomeric repeats



Huppert J. *Chem Soc Rev* (2008)

Location of G-quadruplexes

- Genome-wide sequence analysis identified **376 000** putative sequences *(not homogenously distributed)*

- Within the nucleus:

  a) promoter region of genes

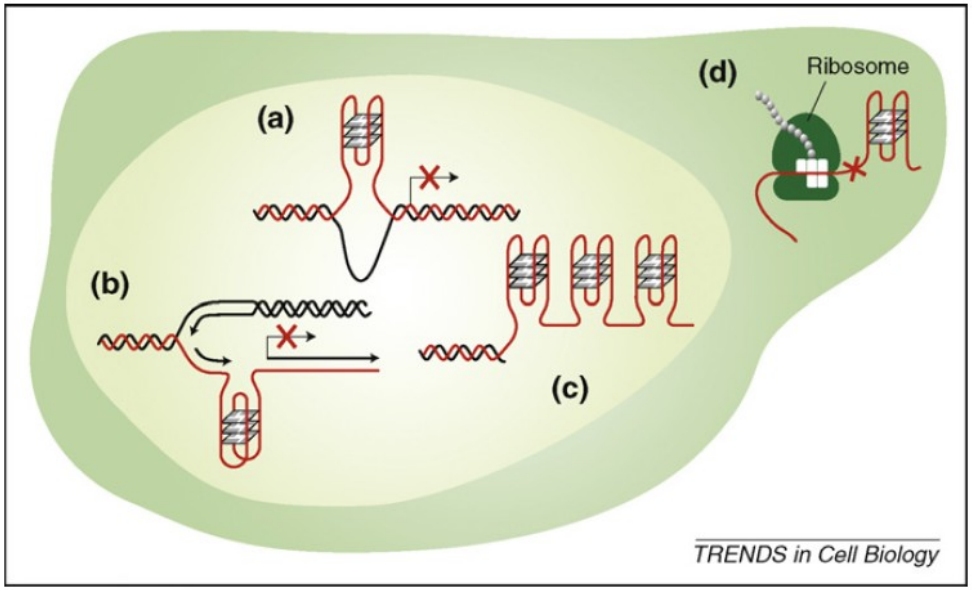
  b) during replication

  c) **telomeric 3'**

  G-overhangs

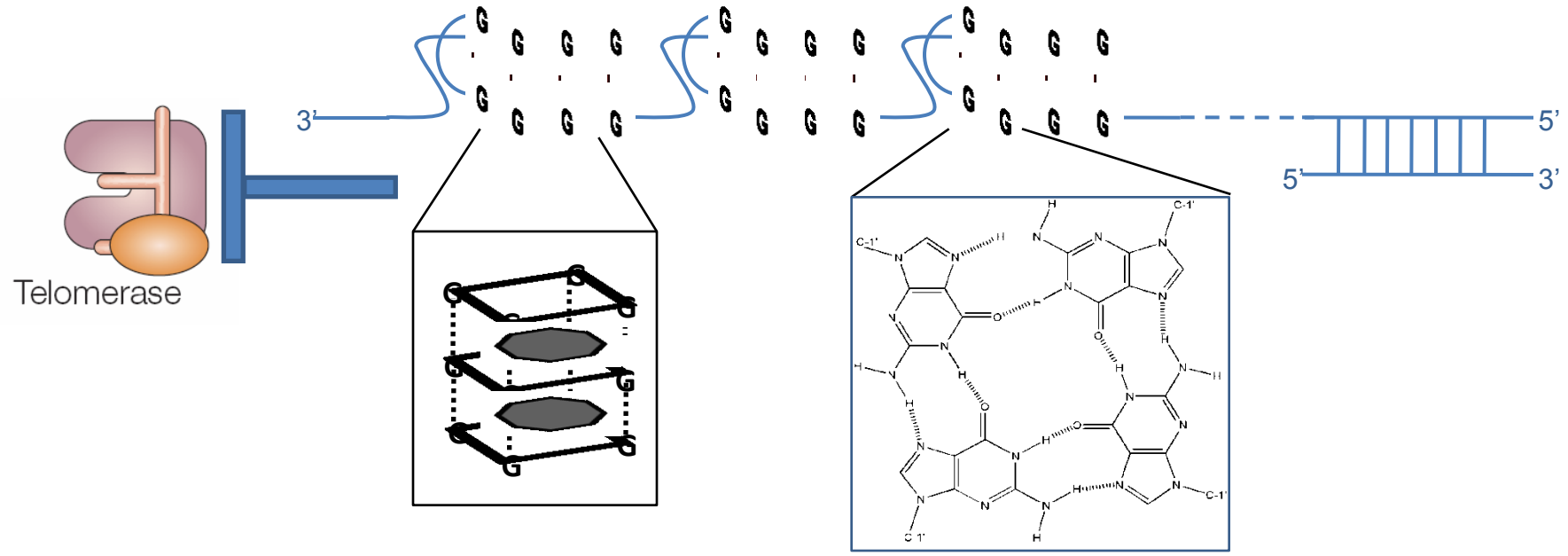
- Outside of nucleus:

  d) 5' UTR of mRNA



Lipps & Rhodes. *Trends in Cell Biology* (2009)

G-Quadruplex Stabilization Leads to Telomerase Repression



Adapted from Fakhoury, J, Nimmo, G, Autexier, C. Anticancer Agents in Medicinal Chemistry, 2007

Phenotypes elicited by G-quadruplex ligands

- Telomerase inhibition
- Telomere shortening
- Lag phase dependent antiproliferative response in cancer cells

- Rapid telomere shortening-independent antiproliferative effects
- Loss of the G-rich overhang
- Dissociation of TRF2 and Pot1 from the telomere
- Increased DNA damage foci at telomeres
- Activation of DNA damage response
- Apoptosis
- Antitumor activity in mouse tumor models

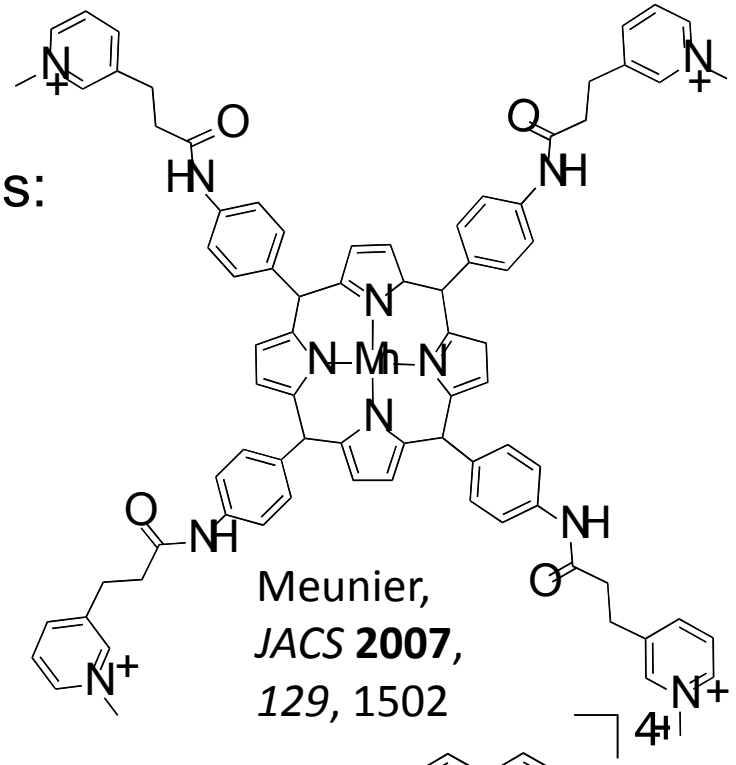
- Antiproliferative response restricted to cancer cells

Reviewed in De Cian, A. et al. Biochimie, 2008.

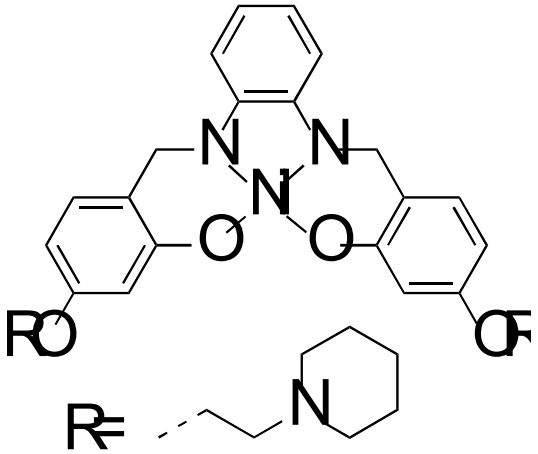
Transition metal-based G-quadruplex binders

Many advantages to implementing metals:

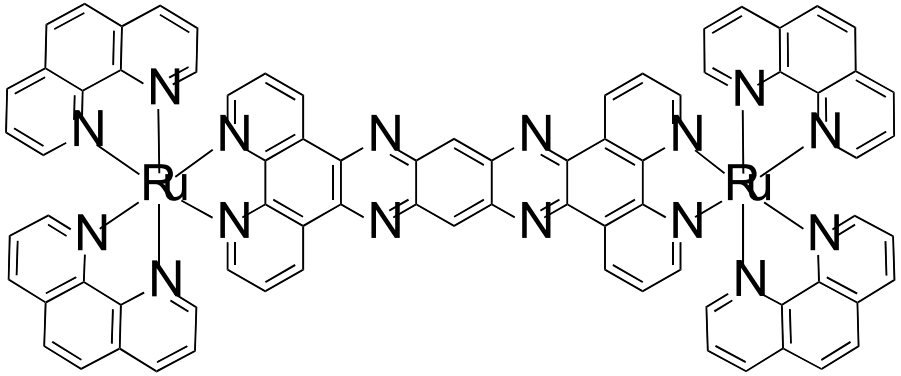
- Modular/tunable
- Multiple metal geometries/inherent positive charge
- Ease of synthesis
- Potential reduction in overall synthetic time



Meunier, *JACS* **2007**, *129*, 1502



Neidle, Vilar, *JACS* **2006**, *128*, 5992



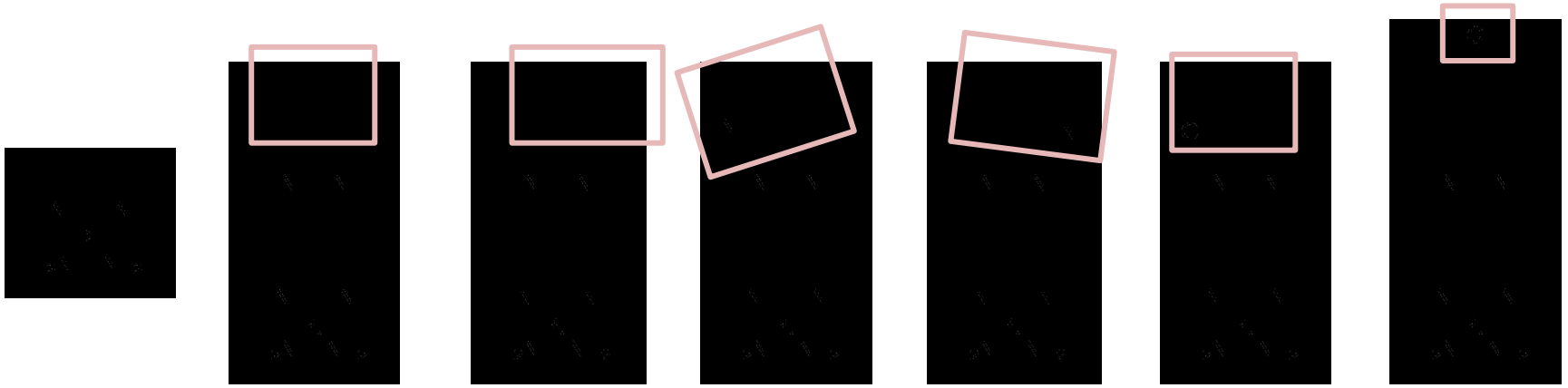
Haq, Thomas, *JACS* **2006**, *12*, 4611

Platinum phenanthroimidazoles as G-quadruplex binders

Challenge: Make DNA binders selective for G-quadruplexes using square planar transition metals to extend the π-surface

R. Kieltyka, Fakhoury J., Moitessier N., Sleiman H., *Chem. Eur. J.,* 2008, *14*, 1055.

Q-quadruplex Ligands



affinity and selectivity to G-quadruplex over duplex DNA

platinum(II)

New class

Added a Halogen, shifting electron density towards Cl
Increased binding affinity and selectivity

Ligand Binding Affinity & Selectivity

- Binding Affinity assessed by FID

  fluorescence intercalator displacement assay

- Compared to 17mer & 26mer

- Binding affinity measured by $^{ds}DC50/^{G4}DC50$

DC 50 concentration of cmplx req to give 50% decrease in fluorescence

PIP
PIN
PII
PIQ
SIP
CLIP
BPY

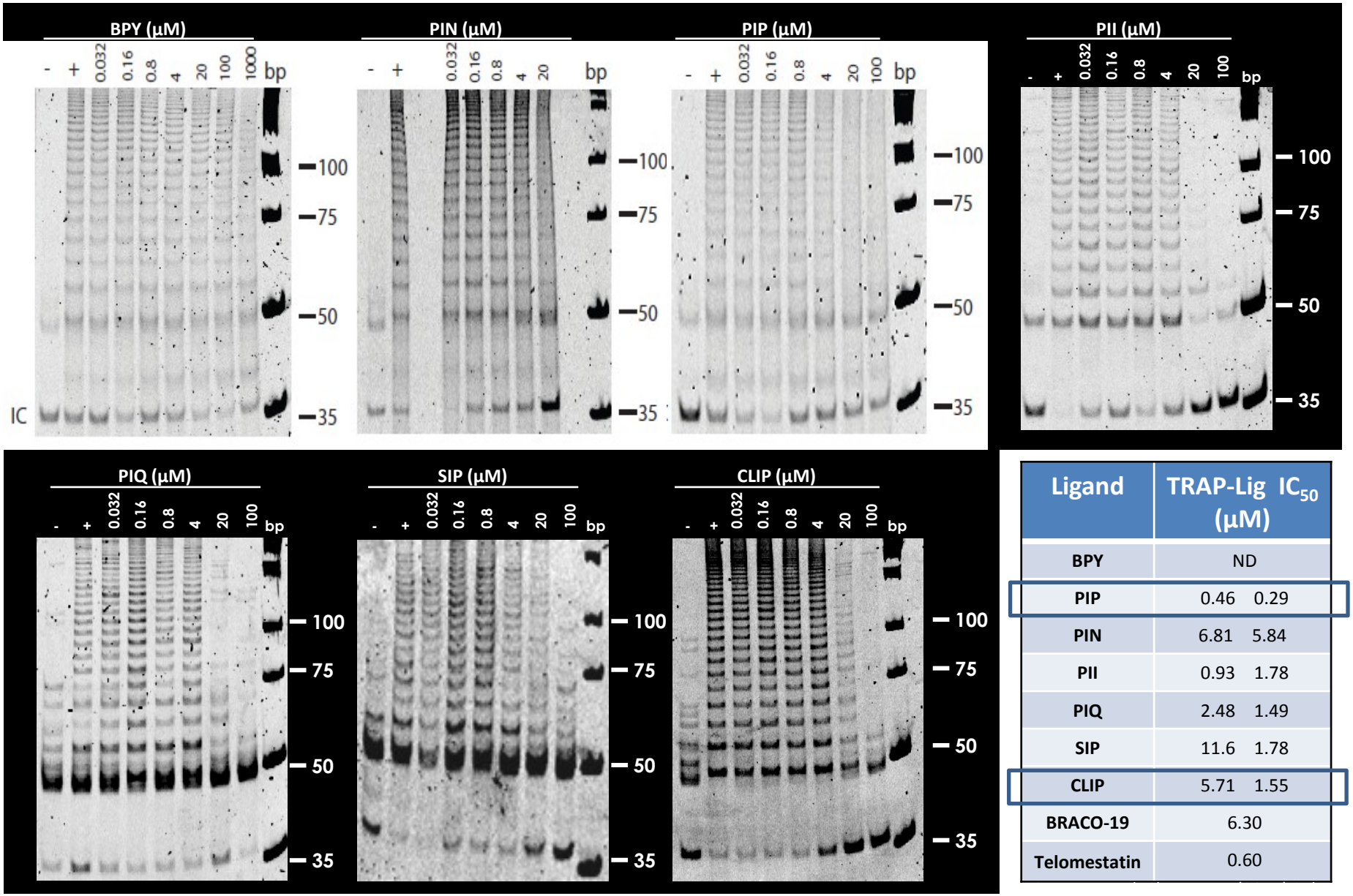
Table 1. DC_{50} values determined from FID assays.

Complex	$^{G4}DC_{50}$ [μM]		$^{ds}DC_{50}$ [μM]		Selectivity[b]
	Na^+	K^+	17mer	26mer	
1	0.66	0.68	1.11	0.84	1.2–1.7
2	1.30	1.71	2.12	1.58	0.92–1.6
3	0.53	1.55	1.37	1.03	0.66–2.6
4	0.70	1.09	1.65	1.37	1.3–2.4
5	0.53	0.70	1.60	1.10	1.6–3.0
6	0.31	0.66	1.47	1.20	1.8–4.7
7	>2.5	ND[a]	>2.5	>2.5	–

[a] Not determined: given the inability of complex **7** to bind strongly to G4 DNA, no thiazole orange displacement was observed, and therefore no DC_{50} value could be determined with the G4 structures formed in potassium-containing buffer. [b] Selectivity range determined from dividing the lowest $^{ds}DC_{50}$ by the highest $^{G4}DC_{50}$ and the highest $^{ds}DC_{50}$ by the lowest $^{G4}DC_{50}$ for each complex.

Castor K et al. ChemMedChem (2012)

In vitro Telomerase Inhibition



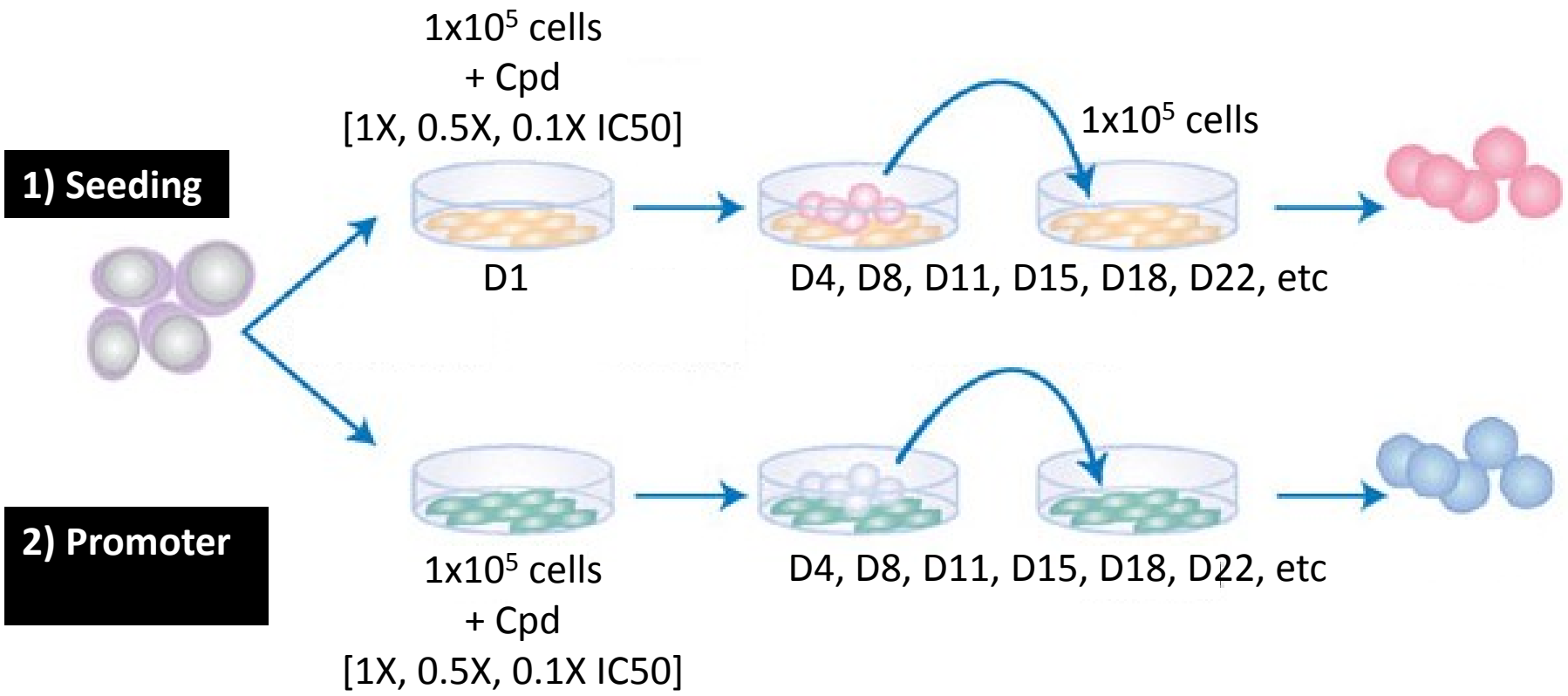
Ligand	TRAP-Lig IC_{50} (μM)
BPY	ND
PIP	0.46 0.29
PIN	6.81 5.84
PII	0.93 1.78
PIQ	2.48 1.49
SIP	11.6 1.78
CLIP	5.71 1.55
BRACO-19	6.30
Telomestatin	0.60

Short term cytotoxicity assay suggest cancer cell specific effects

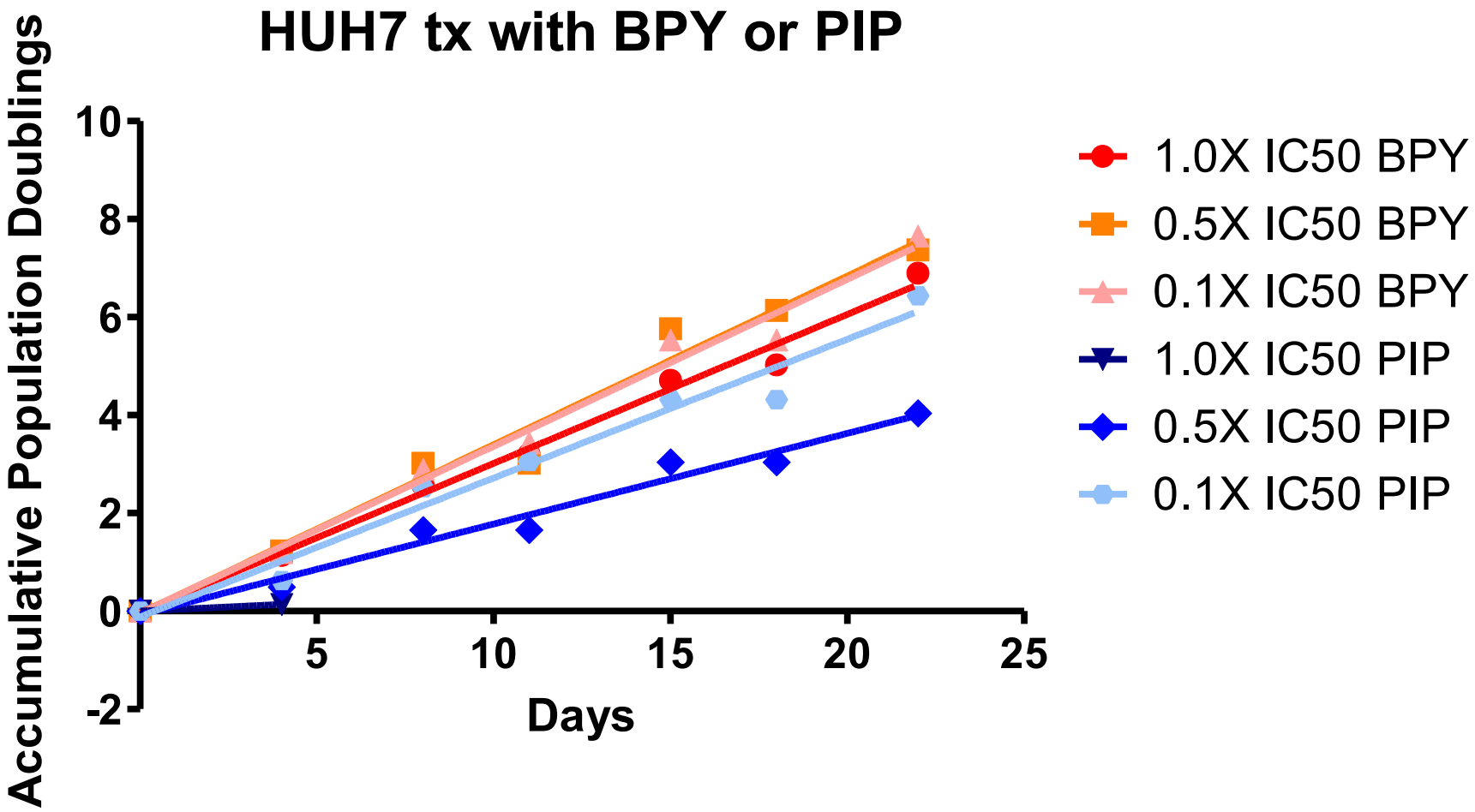
MTS (72h) – Proliferation		IC $_{50}$		
Cell Type	**Cell Line**	**BPY (µM)**	**PIP (µM)**	**CLIP (µM)**
Cancer Telomerase +	**A549**	N/A	21.91 1.20	13.41 1.23
	HUH7	N/A	11.45 1.09	16.70 1.14
	MCF7	N/A	42.91 1.17	18.37 1.11
Cancer, ALT Telomerase -	**GM847**	N/A	27.73 1.04	9.33 1.02
Normal Primary	**MRC-5**	N/A	50.00	57.61 2.85
	WI-38	N/A	70.85	53.63

MTS: Metabolic activity assay

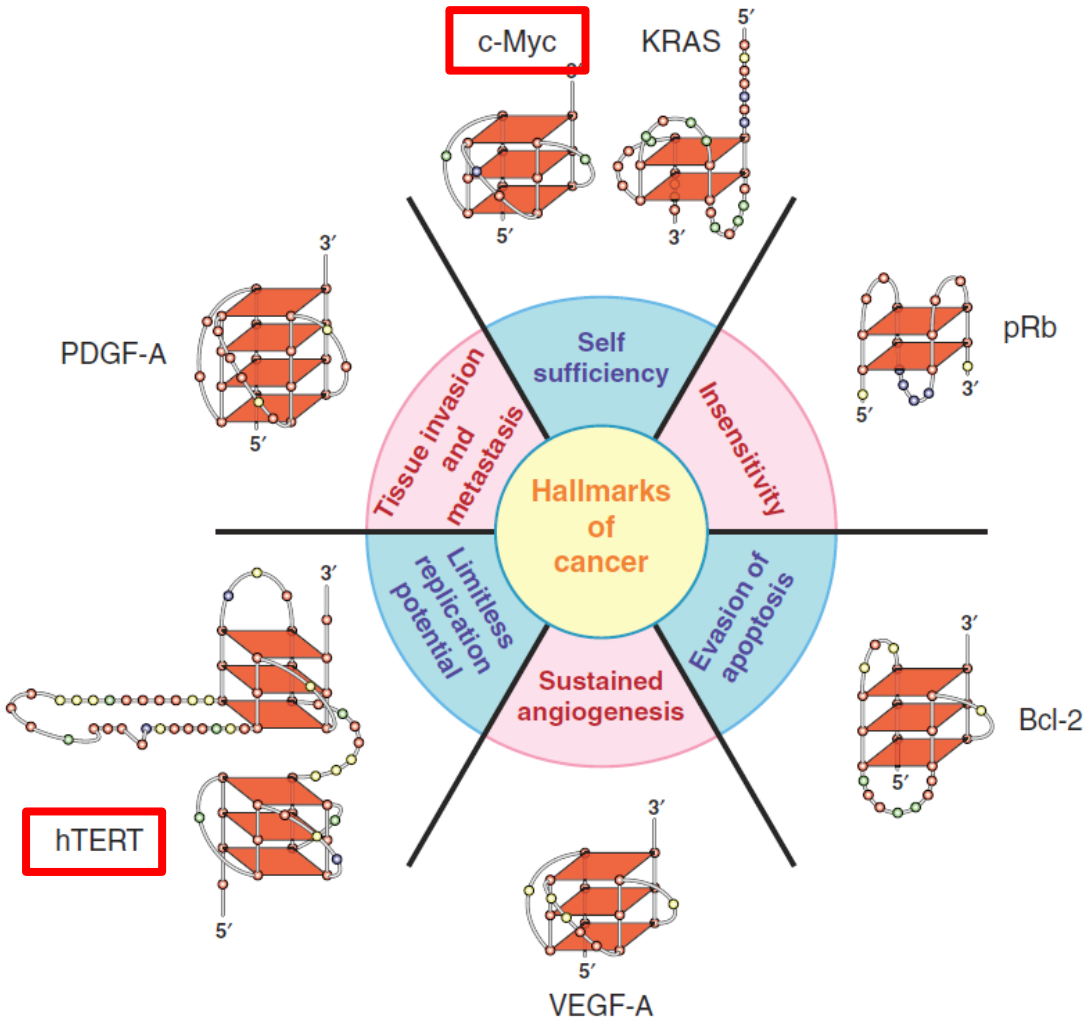
Cell Studies: Experimental Design



Cell Studies: Seeding Experiment



Cell Studies: qPCR



- **c-myc**
  - most studied G4-forming promoter region
  - upregulated in many cancers
  - is a regulator of hTERT
- **hTERT**
  - forms 2 different G4 in the promoter region
  - expression is required for telomerase activity

Conclusions and perspectives

•Molecules with distinct structural features that target G-quadruplex can be generated using supramolecular self-assembly

•phenanthroimidazole platinum(II) complexes are G-quadruplex stabilizers and telomerase inhibitors

•We will further evaluate improved ligands with increase binding affinity and selectivity to the G-quadruplex substrate (telomeric or promoter)

•Ligands are currently being tested for cancer cell specific antiproliferative effects

•Mechanism of action to be determined: telomere length dependent or independent, effect on G-quadruplex containing promoters relevant to telomerase (c-myc, hTERT)

•Best molecule will be tested for antitumor activity in xenograft mouse model

Acknowledgements

Collaborators and Colleagues

Hanadi Sleiman
José-Arturo Londoño-Vallejo
Sun Young Rha
Titia de Lange

Lab members

Shusen Zhu
Yasmin D'Souza
Marie-Eve Brault
May Shawi
Catherine Lauzon
Sanjida Khondaker
Johanna Mancini
Josephine Chu
Nahid Golabi
Ricky Kwan

Alumni

Maria A. Cerone
Johans Fakhoury

Canadian Cancer Society / Société canadienne du cancer
Fonds de la recherche en santé Québec
CIHR IRSC