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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 1

What is Cancer?

"Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death."

The American Cancer Society

How does Cancer Arise?

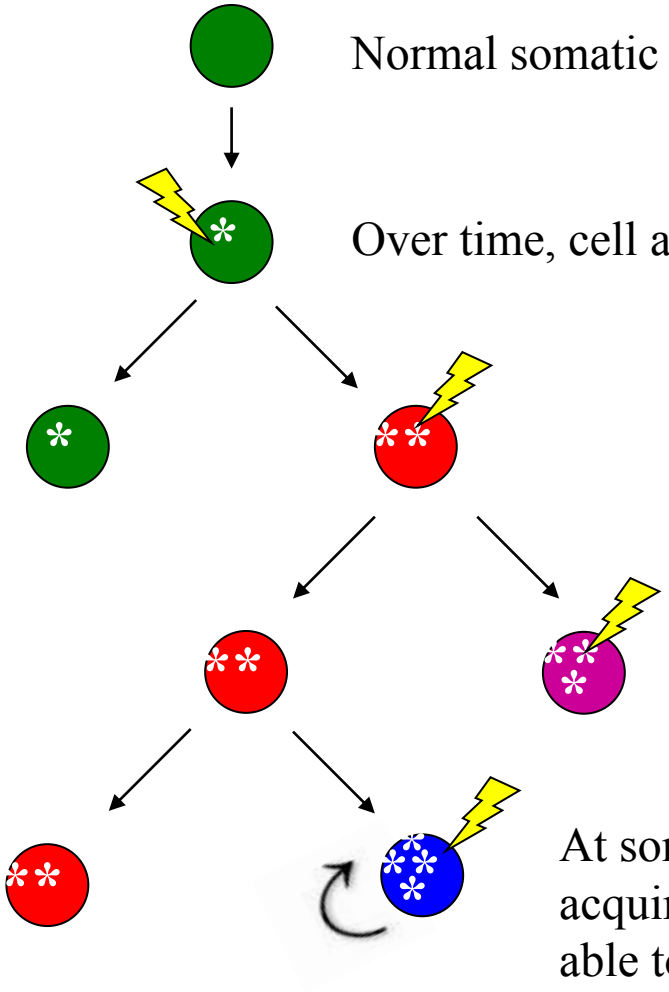
- Through the accumulation of numerous genetic or epigenetic changes within a cell, which together, impart a growth advantage

- Currently, two models of cancer formation are considered

    1) clonal evolution model

    2) cancer stem cell hypothesis

Clonal Evolution Model



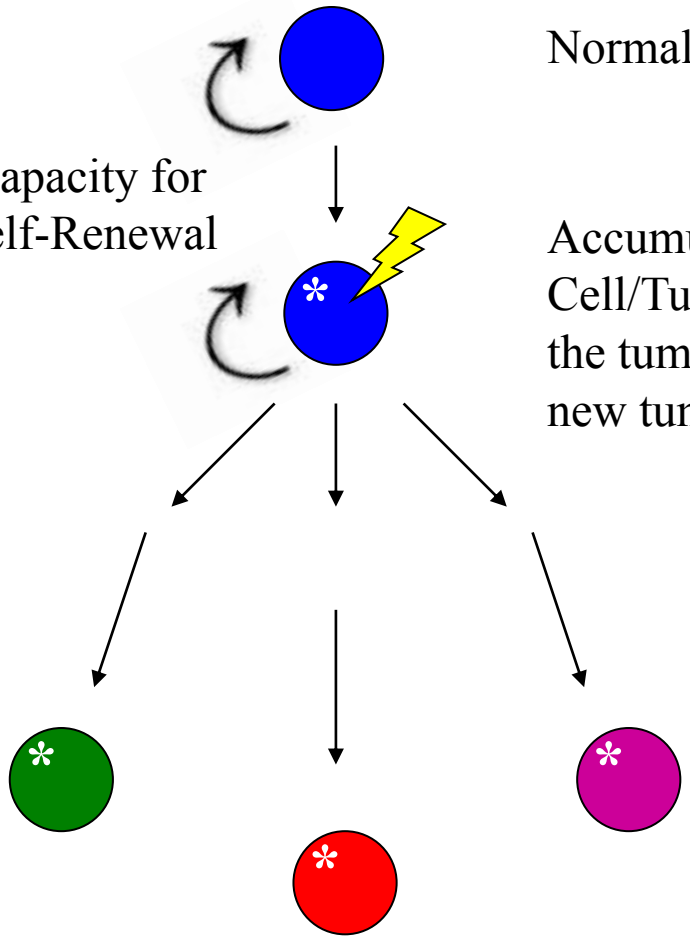
Normal somatic cell within a tissue

Over time, cell accumulates enough mutations to become cancerous

Increased proliferation of tumor leads to the clonal expansion of cancer cells that harbor unique mutations – which confer unique phenotypes onto the cells that carry them. This accounts for the heterogeneity that is characteristic of many cancers.

At some point, a certain population within the tumor could acquire the ability for self-renewal – these cells would be able to form a new tumor if transplanted into a new host.

Stem Cell Hypothesis for Cancer

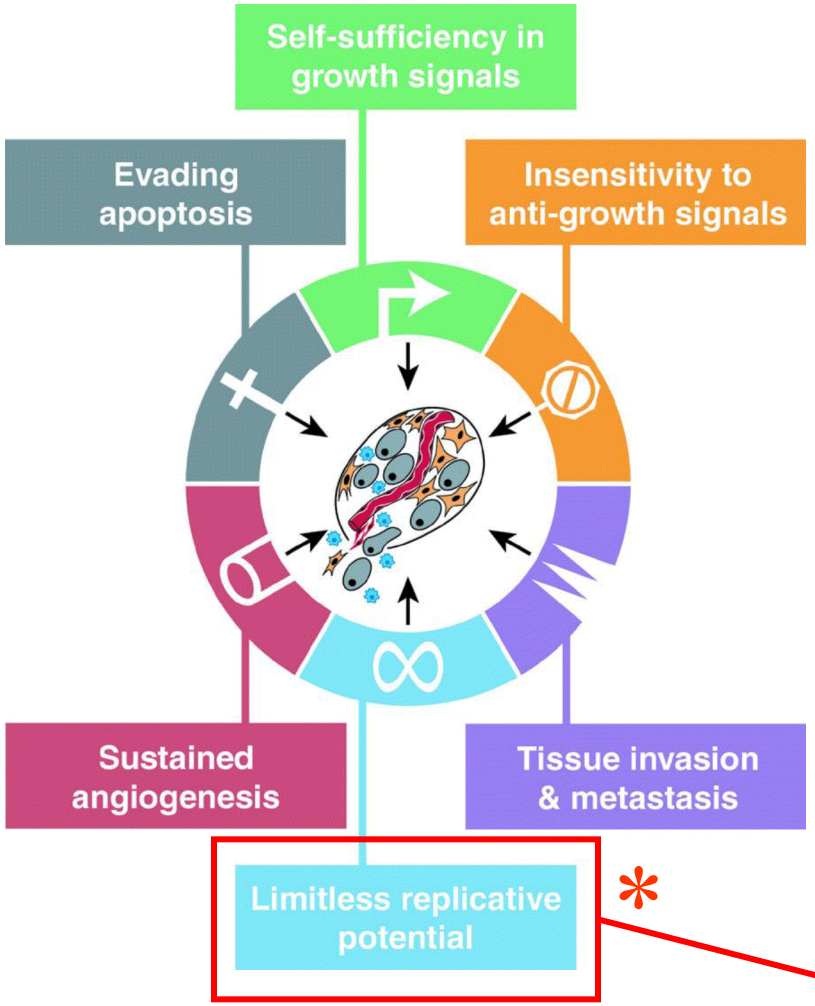


Normal stem cell – gives rise to various cell types within a tissue:

Accumulation of multiple mutations over time – Cancer Stem Cell/Tumor Initiating Cell (considered a small proportion of the tumor mass – these are the only cells capable of forming a new tumor or giving rise to metastases)

The majority of the tumor is composed of cancer cells that do not possess stem cell/tumor initiating cell properties. They may acquire additional mutations beyond those originally found in the CSC that gave rise to them. However, none of these cells can form a new tumor if transplanted into another host.

Phenotypic Characteristics of Cancer Cells



Most cancers must acquire several "functions" in order to progress from their normal cellular origins to invasive carcinoma

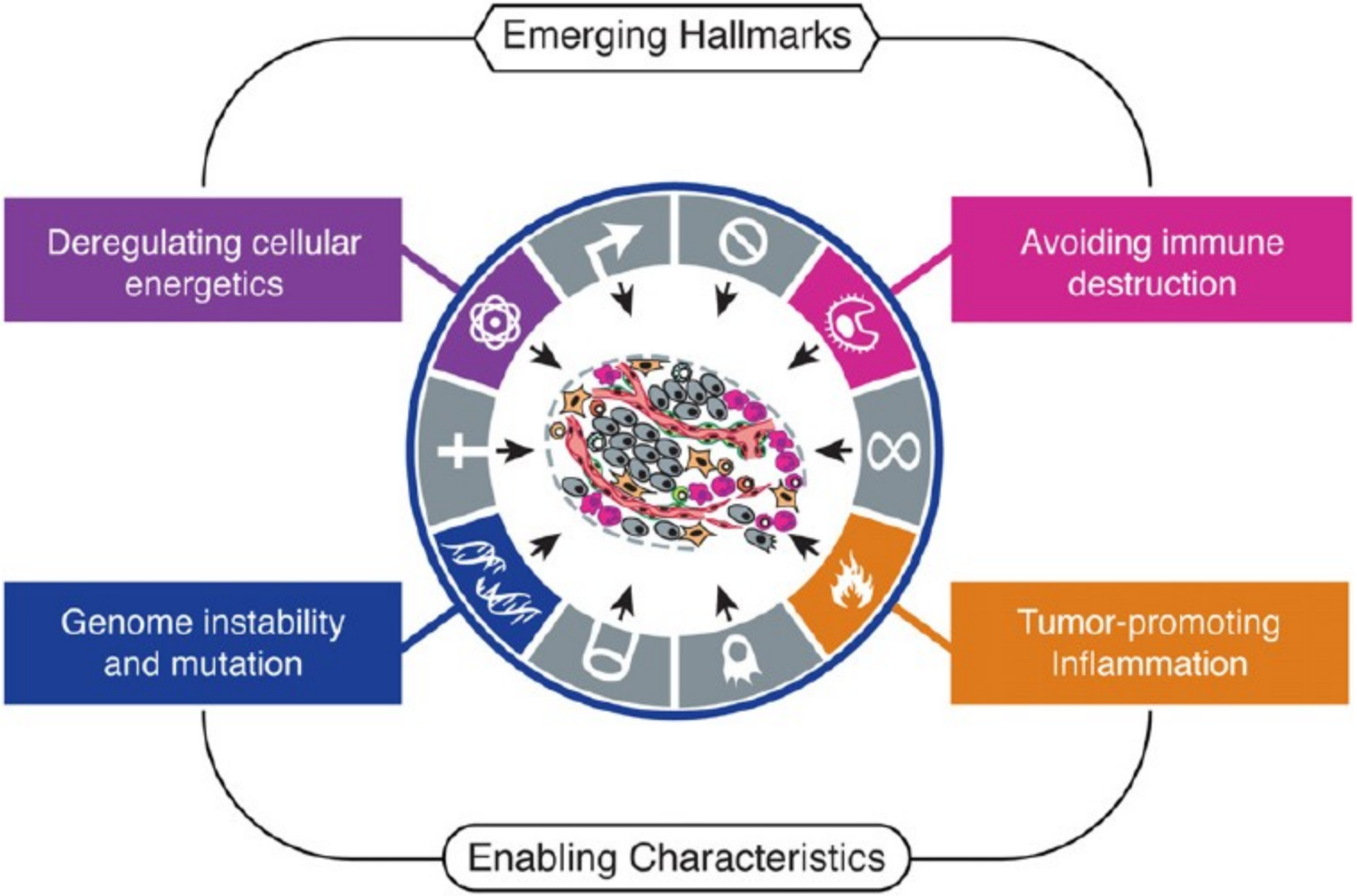
The molecular events (mutation, chromosome loss/gain, translocations) that underlie these functions can be different between different types of cancer

The acquisition of these alterations within a "prospective" cancer cell are sequential and may account for the fact that many cancers arise later in life

Critical Role for Telomere Maintenance

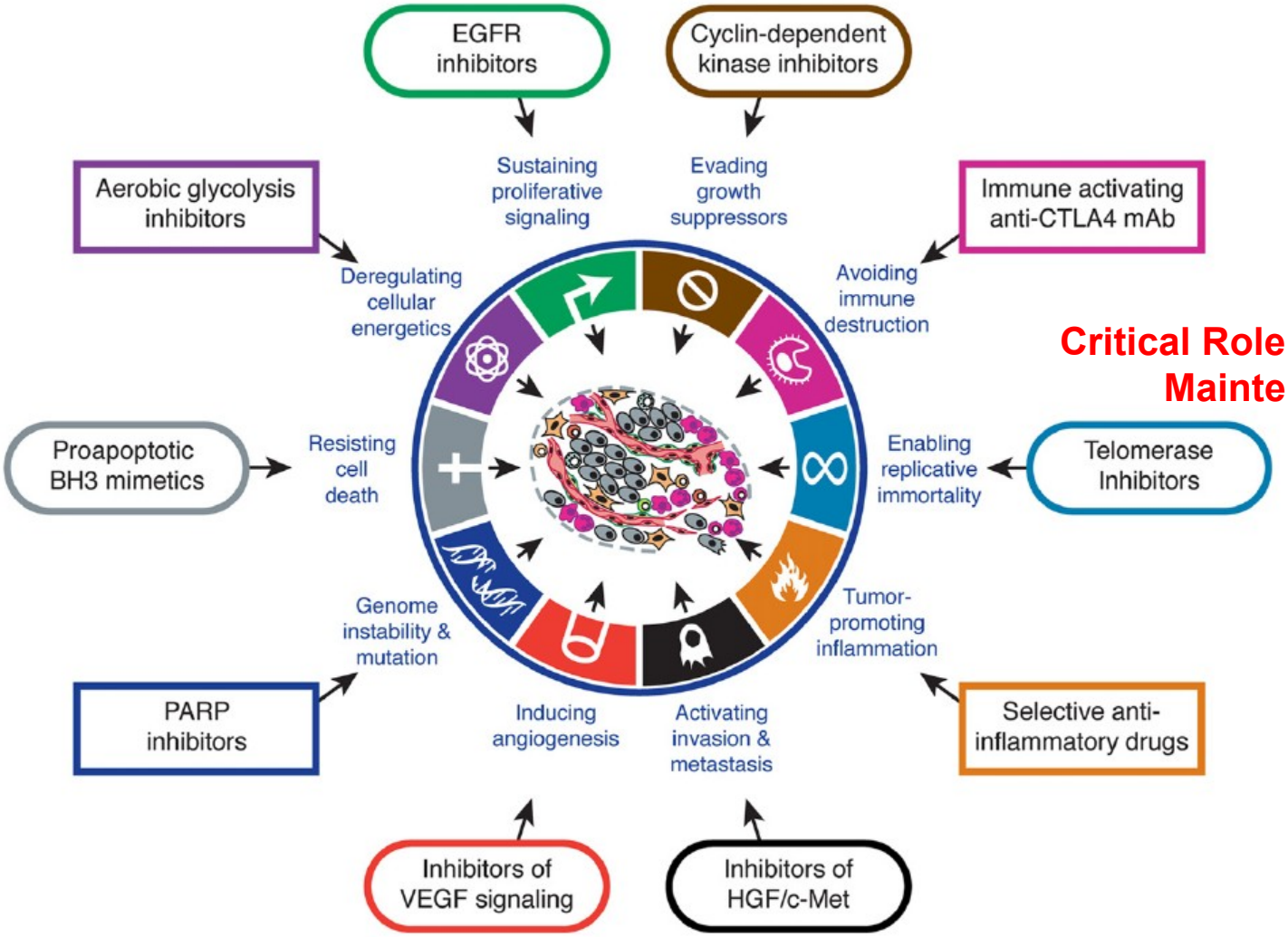
Hanahan and Weinberg (2000). Cell 100(1), 57-70.

Emerging Hallmarks and Enabling Characteristics



Hanahan and Weinberg (2011). Cell. 144, 646-674.

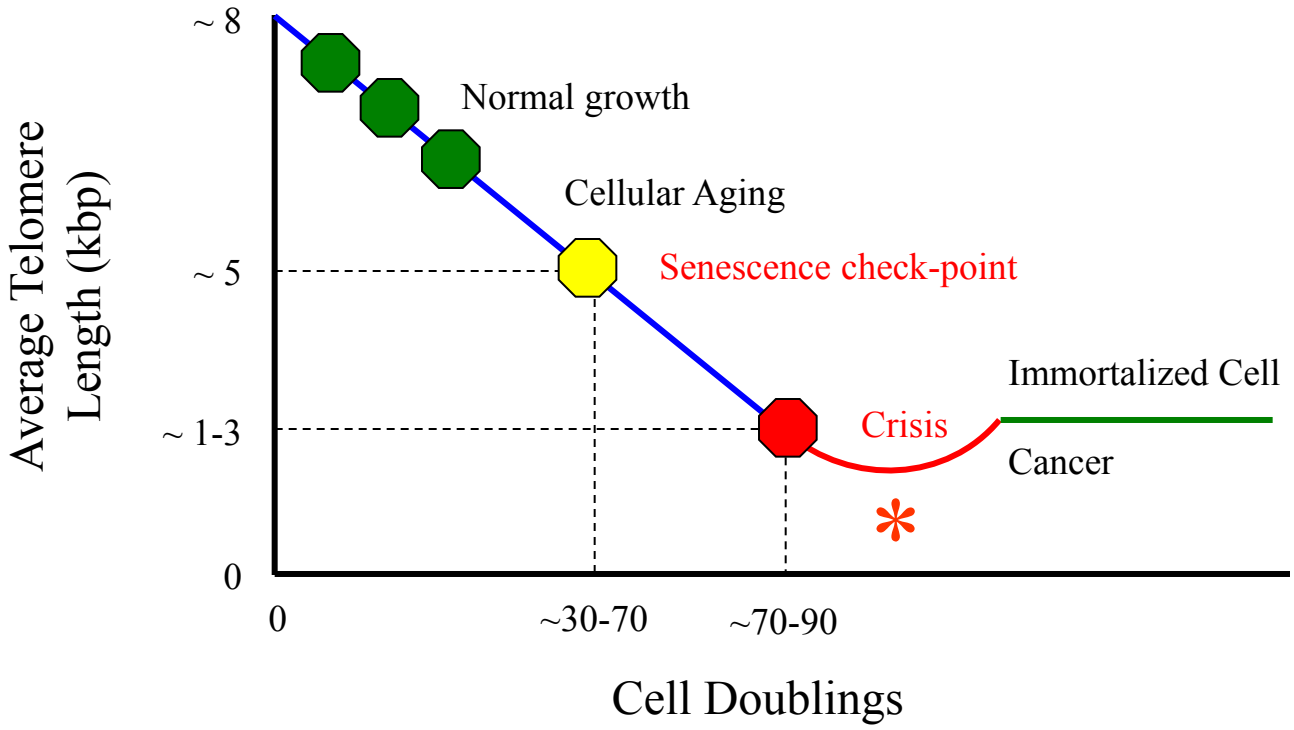
Therapeutic targeting of the hallmarks of cancer



Critical Role of Telomere Maintenance

Hanahan and Weinberg (2011). Cell. 144, 646-674.

How Does Telomere Maintenance Affect Replication Potential?



Modified from Harley (2008). Nat Rev Cancer 8: 167-179

Checkpoints Controlling Cell Growth

DNA damage signal - critically short telomere → Activate DNA damage response Kinase (ATM/ATR) → Activate downstream substrates **Eg. p53**

Cell Cycle Control
Transient Growth Arrest or Senescence
p21

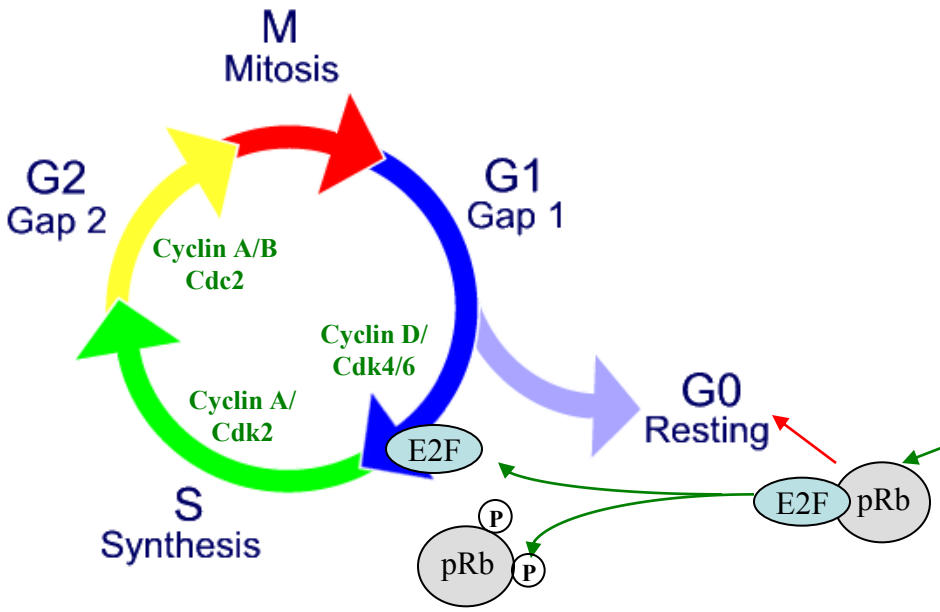
Cdk Inhibitors

p15	p21
p16	p27
p18	p57
p19	

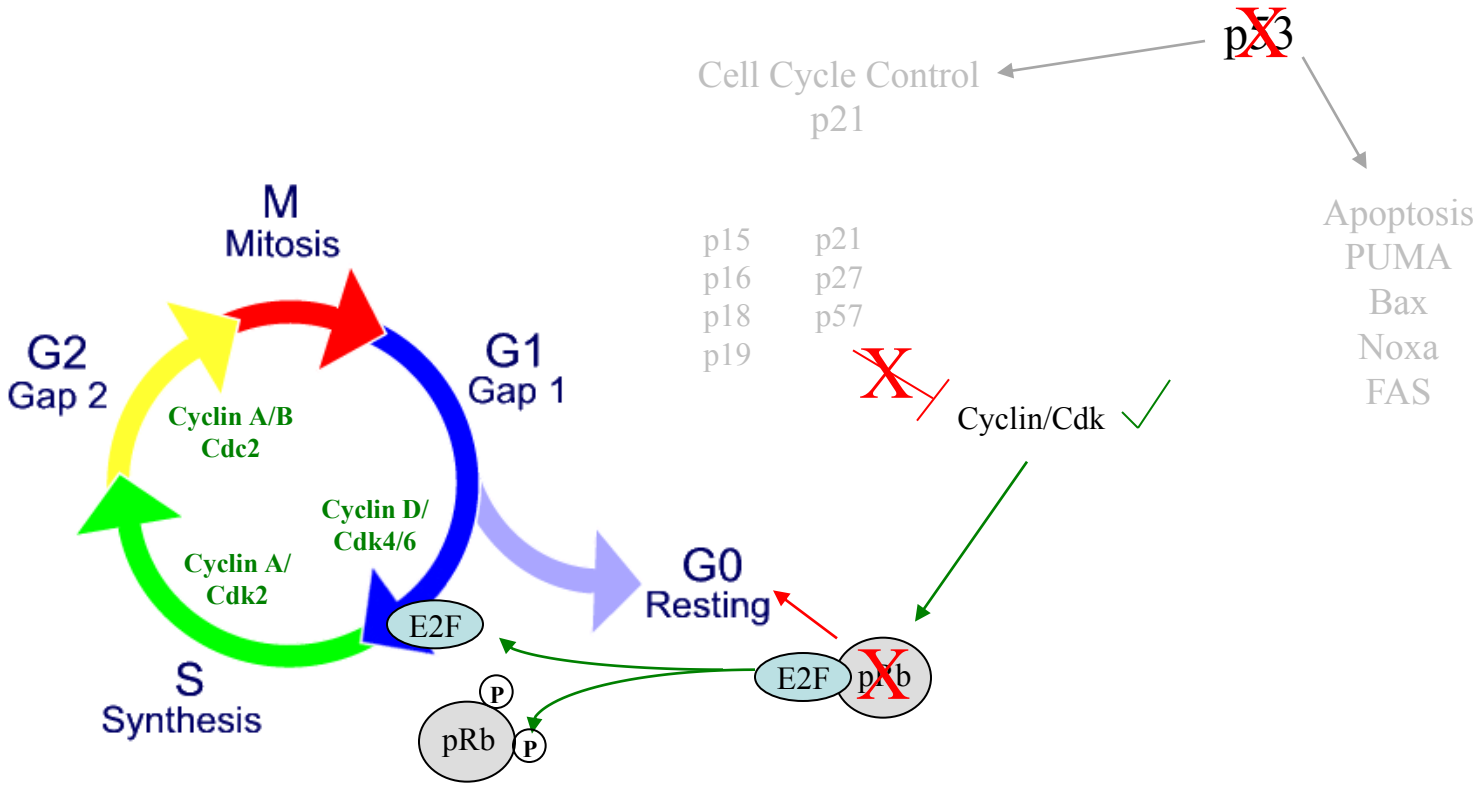
Cyclin/Cdk

Apoptosis
PUMA
Bax
Noxa
FAS

Elimination of cells that are beyond repair

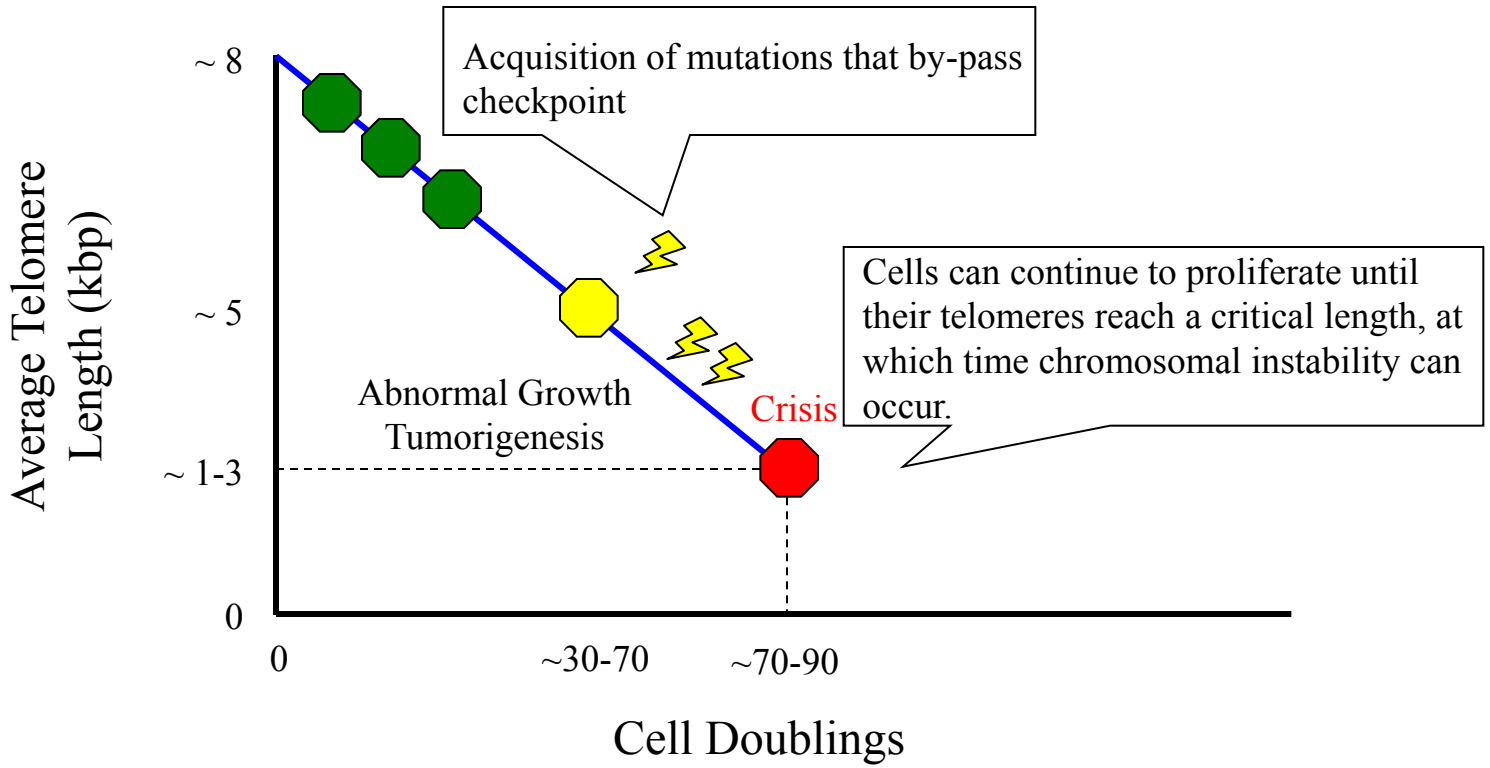


How Can Checkpoints be By-passed?



1) Loss of p53, pRb (tumor suppressor genes) X
2) Loss of Cdk inhibitors X
3) Overexpression of cyclin/Cdk complexes ✓
4) Expression of Oncogenes (Ras, Myc)

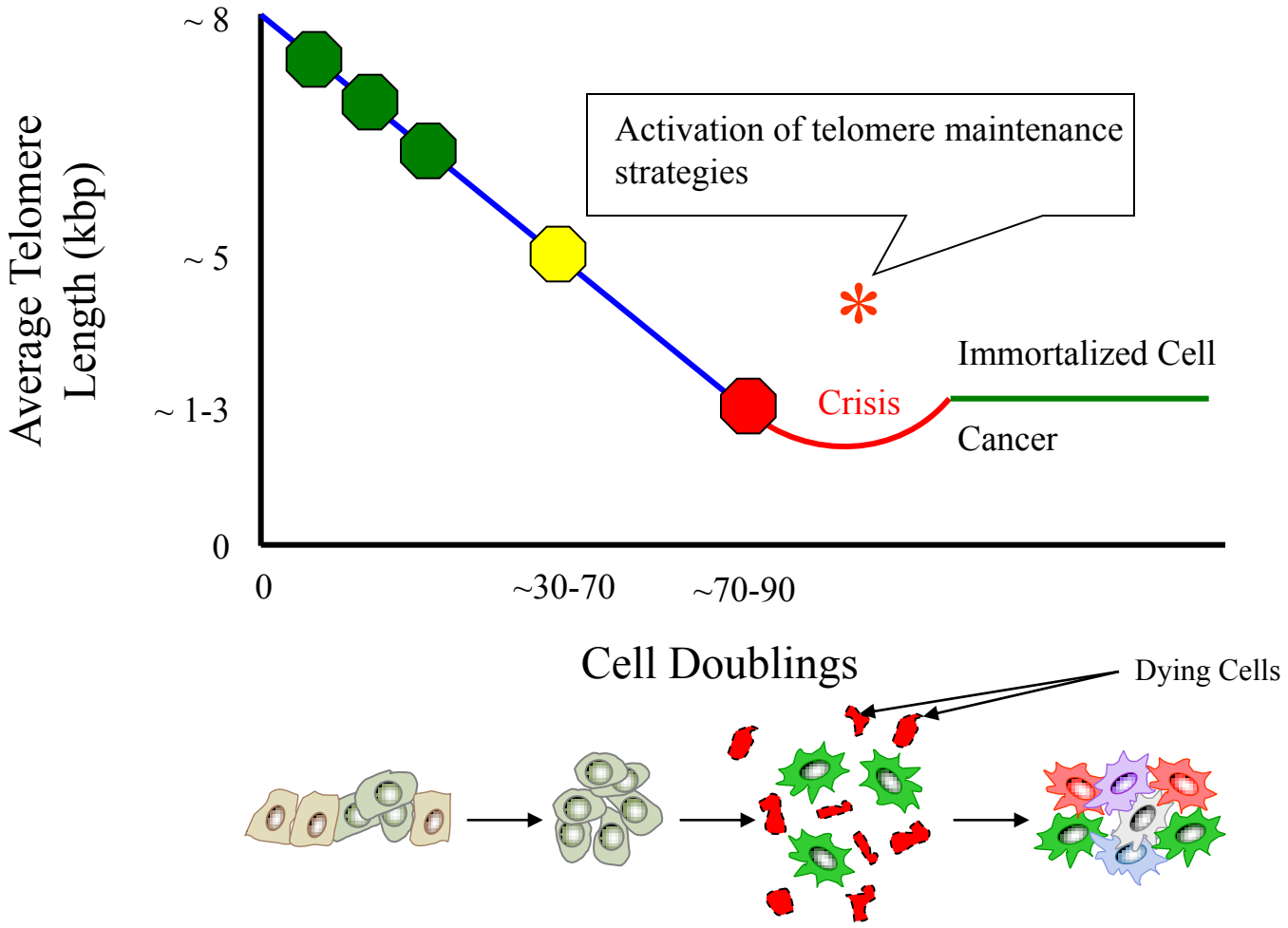
Crisis



The majority of cells undergoing crisis due to critically short telomeres will undergo apoptosis due to the fact that the chromosomal rearrangements are incompatible with cell viability.

Cells must stabilize their telomeres in order to survive crisis. The period of genomic instability can promote the emergence of malignant cells due to the accumulation of mutations, gene amplifications, gene fusions or gene deletions

Telomere stability/Telomerase reactivation is a Key Characteristic of Cancer Cells

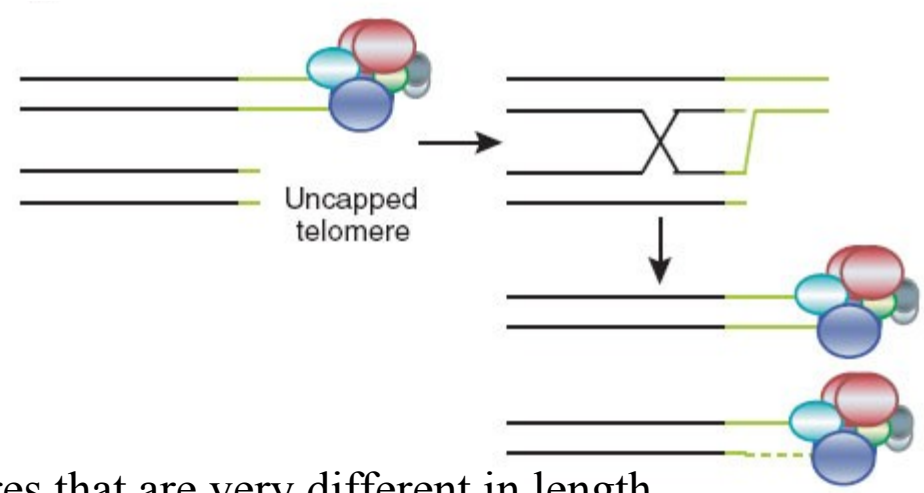


Strategies for Telomere Length Maintenance
ALT Mechanism

ALT- Alternative Lengthening of Telomeres

Characterized by heterogeneous telomere lengths, presence of PML-bodies (regions in the nucleus that contain telomeric DNA, proteins involved in DNA repair and recombination.)

Mechanism based on inter-chromosomal recombination



Results in telomeres that are very different in length

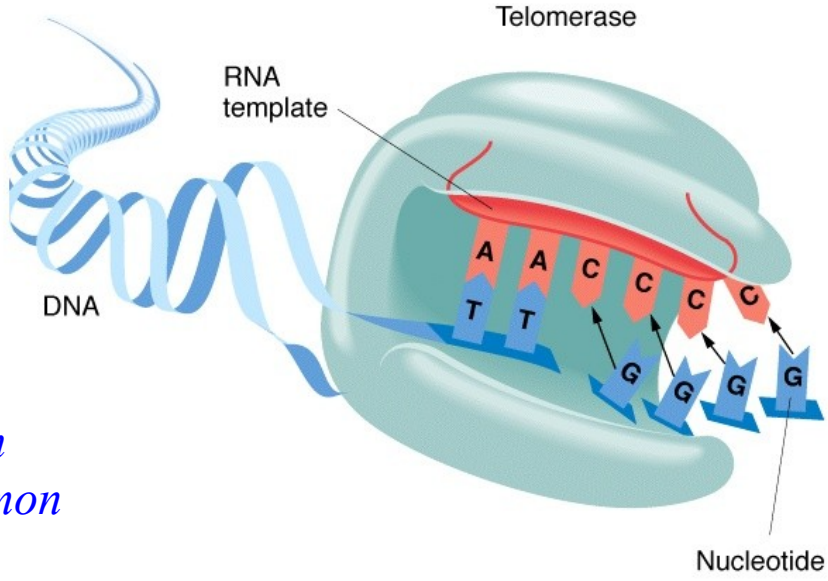
Cancers and cancer derived cell lines that display an ALT phenotype are typically derived from mesenchymal tissues

Strategies for Telomere Length Maintenance
Telomerase Activation

Telomerase mediated Telomere Maintenance

Activity of the Telomerase Complex – minimally composed of TERT (the telomerase reverse transcriptase) and the telomerase RNA template (TR, TER, TERC)

Upon activation, extends telomeric repeats at chromosomal ends (telomere lengths are more homogeneous)



Telomerase activation is typically observed in epithelial cancers – represents the most common cancer type in humans

Approximately 80-90% of human cancers display activation of telomerase compared to the remaining 10-20% that display features of ALT

Is Telomerase Activity Required for Transformation?

Evidence from Cell Based Models

Inhibition of Telomerase (expression/function) in cancer cells using dominant-negative hTERT or inhibitors pharmacological inhibitors can induce telomere shortening in cancer cells

Suggests that telomerase activity/telomere integrity is required for the transformed phenotype

Table 2 Effects of DN-hTERT on tumorigenicity in 36M ovarian cancer cells

	Number of tumors/ Number of injections	Population doubling	Mean telomere length (kb)
Vector, c3	6/6	32	5–7
WT-hTERT, c6	6/6	33	6–8
DN-hTERT, c2	0/6	30	ND
DN-hTERT, c6	0/6	34	2–3.5
DN-hTERT, c7	0/6	32	2.5–4.5

Population doubling and mean telomere lengths of cells at the time of injection. ND, not determined; c, clone number.

Mutations in the reverse transcriptase domain – can function as a dominant-negative

Hahn *et al*., (1999). Nature Med 5, 1164-1170.

Is Telomerase Activity Sufficient for Transformation?

Evidence from Cell Based Models

Transformation of primary human epithelial cells can be accomplished by the introduction of hTERT, SV40 Large T/small t, and Ras

hTERT expression alone is not sufficient for transformation

Telomerase should not be considered an oncogene!

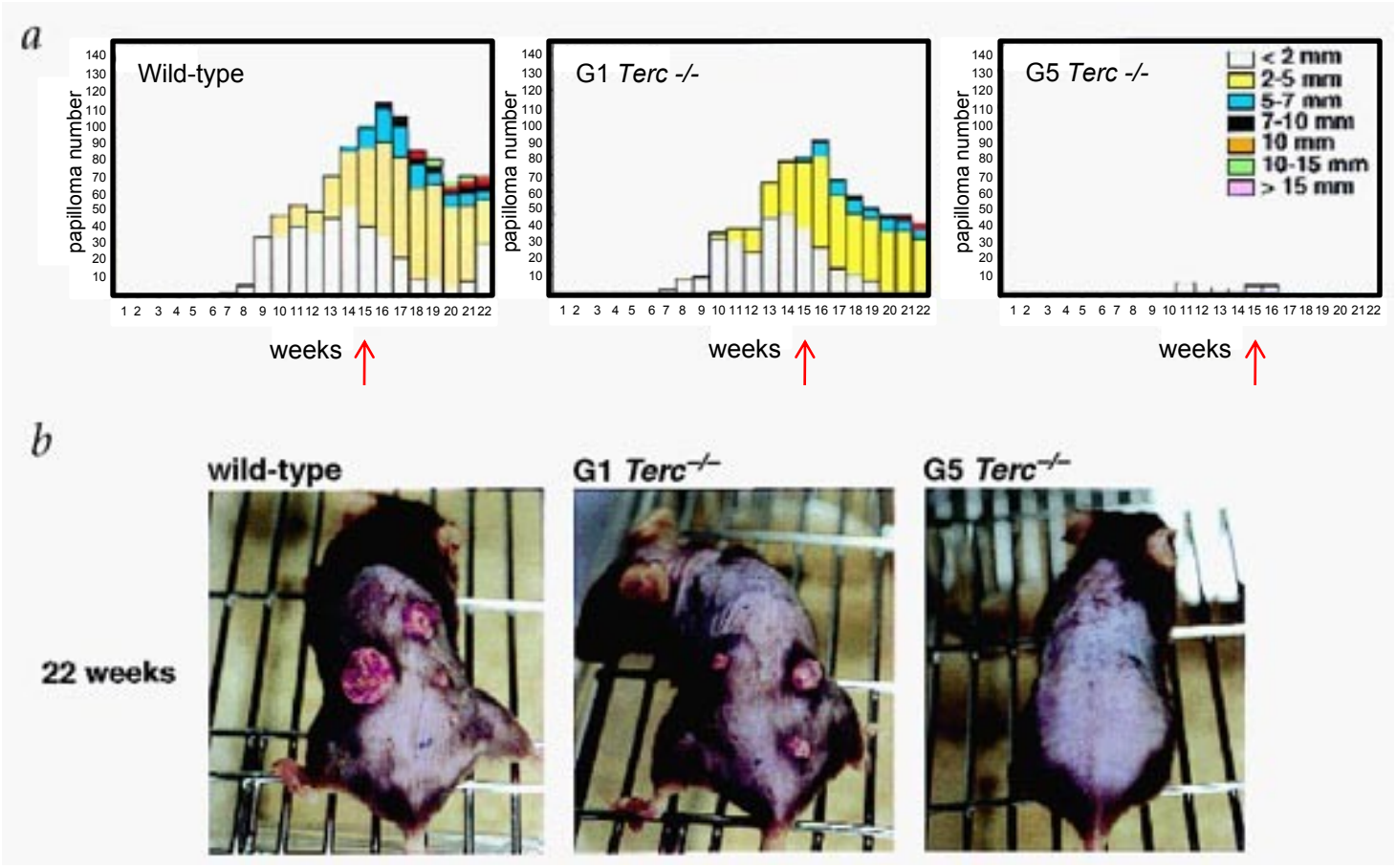
Table 1. *Formation of subcutaneous tumors in nude mice*

Cells	Genotype	No. tumors/ injection	Ras over-expression
HMEC	hTERT, V	0/3	—
	hTERT, Ras-puro	0/6	12.0
	LT, V, V	0/3	—
	LT, hTERT, V	0/6	—
	LT, Ras-puro	0/3	12.0
	LT, hTERT, Ras-hygro	0/24	3.5
	LT, hTERT, Ras-zeo	1/15	7.2
PHMEC	LT, hTERT, V	0/9	0
HEK	LT, hTERT, Ras-hygro	1/7	9.5

Elenbaas *et al*., (2001). Genes Dev 15, 50-65.

Insights from Mouse Models Lacking Telomerase Function

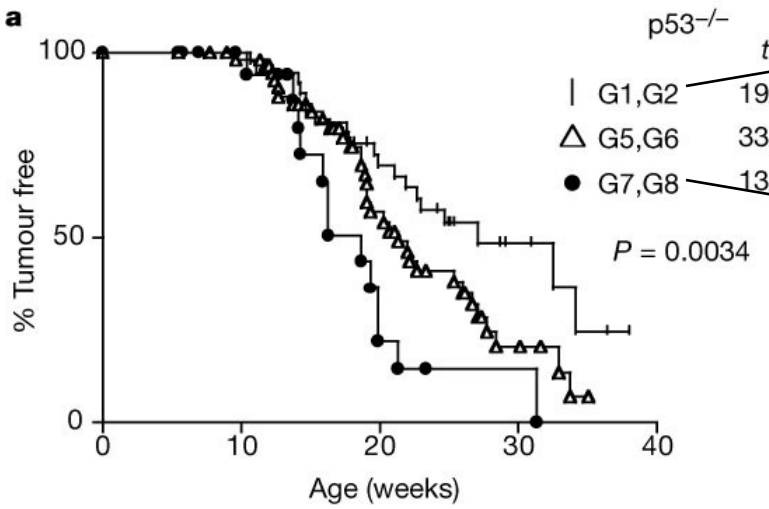
Telomerase Deficient-Mice (Late Generation) are Resistant to Carcinogen-Induced Skin Tumorigenesis



Gonzalez-Suarez *et al*., (2000). Nature Genetics 26, 114-117.

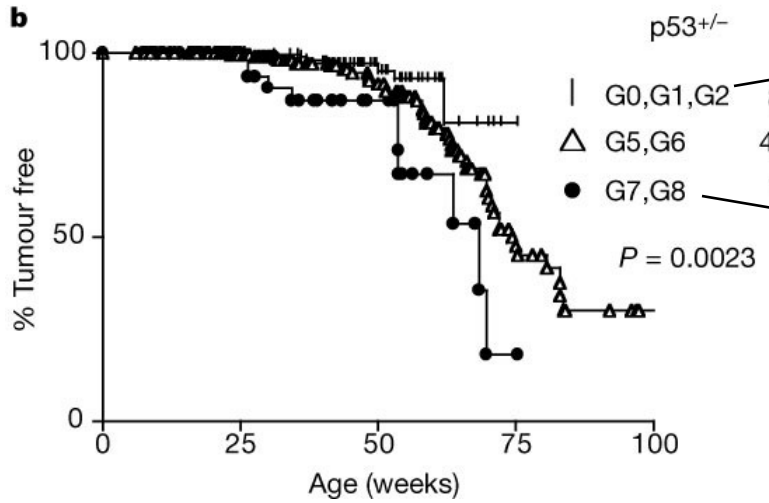
These mice are in a wild-type genetic background – intact p53

Telomerase-Deficiency (Late Generation) in the Context of p53 loss Enhances Tumorigenesis



Majority Sarcomas, Lymphomas
No adenocarcinomas

Majority Sarcomas, Lymphomas
Emergence of Adenocarcinomas (9%)



Sarcomas, Lymphomas
No adenocarcinomas

Majority Adenocarcinomas (55%)
Remaining Sarcomas, Lymphomas (45%)

Majority of these tumors had lost the remaining p53 allele

Artandi *et al*., (2000). Nature 406, 641-645.

Intact checkpoint:
Loss of telomerase results in impaired tumorigenesis

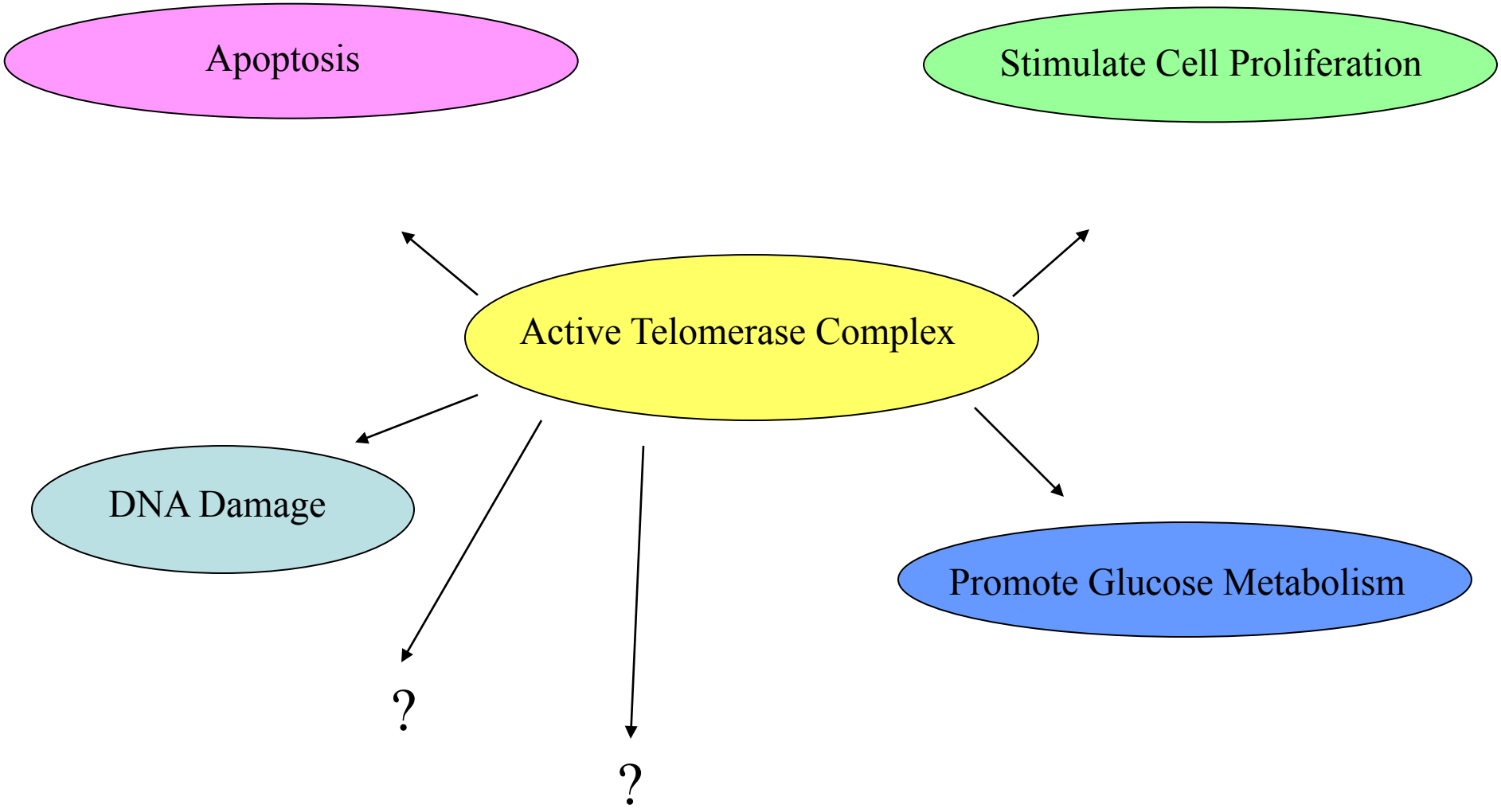
Genotype or treatment	Tumour phenotypes	Effect of dysfunctional telomeres (phenotype in *Terc*$^{-/-}$ background)	Ref
No mutations	Few tumours normally seen	Compared with wild-type and early-generation telomerase-null mice, ageing late-generation mice show an increase in the incidence of cancer	32,42
DMBA/TPA treatment	Treatment with these carcinogens allow for the monitoring of tumour initiation (papillomas) and progression to SCCs of the skin	Loss of telomerase (G1) resulted in decreased growth rate and size of papillomas, with a slight decrease in numbers. G5 mice with dysfunctional telomeres were almost completely resistant to papilloma formation	50
Cdkn2a	Deletion of this locus results in loss of both INK4A and ARF. The resulting mice develop lymphomas and sarcomas	Late-generation double knockouts show decreased incidence of spontaneous and carcinogen-induced tumours, and increased tumour latency	49,93
Apc^{min}	100% of mice with this mutation develop multiple intestinal neoplasias that progress from microadenomas to macroadenomas	Short telomeres led to increased tumour initiation (microadenomas) but decreased size and number of macroscopic adenomas	51
Alb–uPA transgene or CCl_4 or DEN treatment	This transgenic mouse and the carcinogenic treatment are both effective ways of modelling HCC	Successive breeding of *Alb–uPA* onto a late-generation telomerase-null background or treatment of G3/G4 mice with CCl_4 or DEN resulted in decreased number and size of liver nodules	53
Pms2	Deficiency of this mismatch repair gene leads to increased susceptibility for lymphomas, sarcomas and colon carcinomas	Progressively shortening telomeres reduced the incidence of all three tumour types	94
Eμ-Myc	Transgenic expression of *Myc* in B cells leads to potent formation of lymphoma in this model for Burkitt lymphoma	Formation of lymphoma was almost completely suppressed for 2 years in mice with dysfunctional telomeres (G5/G6), unlike wild-type and G1 mice that developed cancer within 6 months	57
Atm	Thymic lymphoma	Delayed onset and decreased incidence of thymic lymphomas	95,96
Trp53	Loss of this important tumour suppressor leads to rapid development of mainly lymphomas and soft tissue sarcomas	Combined homozygous loss of p53 and dysfunctional telomeres led to increased incidence of lymphomas. In addition, late-generation *Terc*$^{-/-}$ combined with heterozygous loss of p53 showed a shift in tumour range from lymphomas to epithelial cancers (breast, GI and SCC) with non-reciprocal translocations	47
p53^{R172P}	This point mutation commonly found in human tumours abolishes the ability of p53 to induce apoptosis and delays tumour formation for about 6 months.	Mice from an intergenerational cross with *Terc*$^{-/-}$ animals (iG1) have dysfunctional telomeres and show almost complete suppression of tumorigenesis	58
Atm and *Trp53*	T-cell lymphoma	Loss of p53 accelerated onset of lymphomas in *Terc*$^{-/-}$ ATM$^{-/-}$ mice	97
K5-Trf2	Specific overexpression of TRF2 in the skin of these mice leads to development of spontaneous SCC on the skin	Increasing generations of telomerase-deficient mice showed accelerated onset of spontaneous and UV-induced skin neoplasms	98

Apc^{min}, adenomatous polyposis coli min (multiple intestinal neoplasia) allele; ATM, ataxia telangiectasia mutated; DEN, diethylnitrosamine; G, generation; GI, gastrointestinal; HCC, hepatocellular carcinoma; K5, keratin 5; PMS2, postmeiotic segregation increased 2; Terc, telomerase RNA component; SCC, squamous cell carcinoma; TRF2, telomeric repeat-binding factor 2; TPA, 12-O-tetradecanoylphorbol-13-acetate; UV, ultraviolet.

Defective checkpoint:
Loss of telomerase results in enhanced tumorigenesis

Deng et al., (2008). Nat Rev Cancer; 8(6):450-458.

Potential Telomerase Functions?



Telomerase Functions in Apoptosis

However, numerous studies point to a role for hTERT in protecting cells from apoptosis that is independent of its catalytic activity and telomere maintenance…

Cao *et al.*, (2002). Oncogene 21:3130–3138.

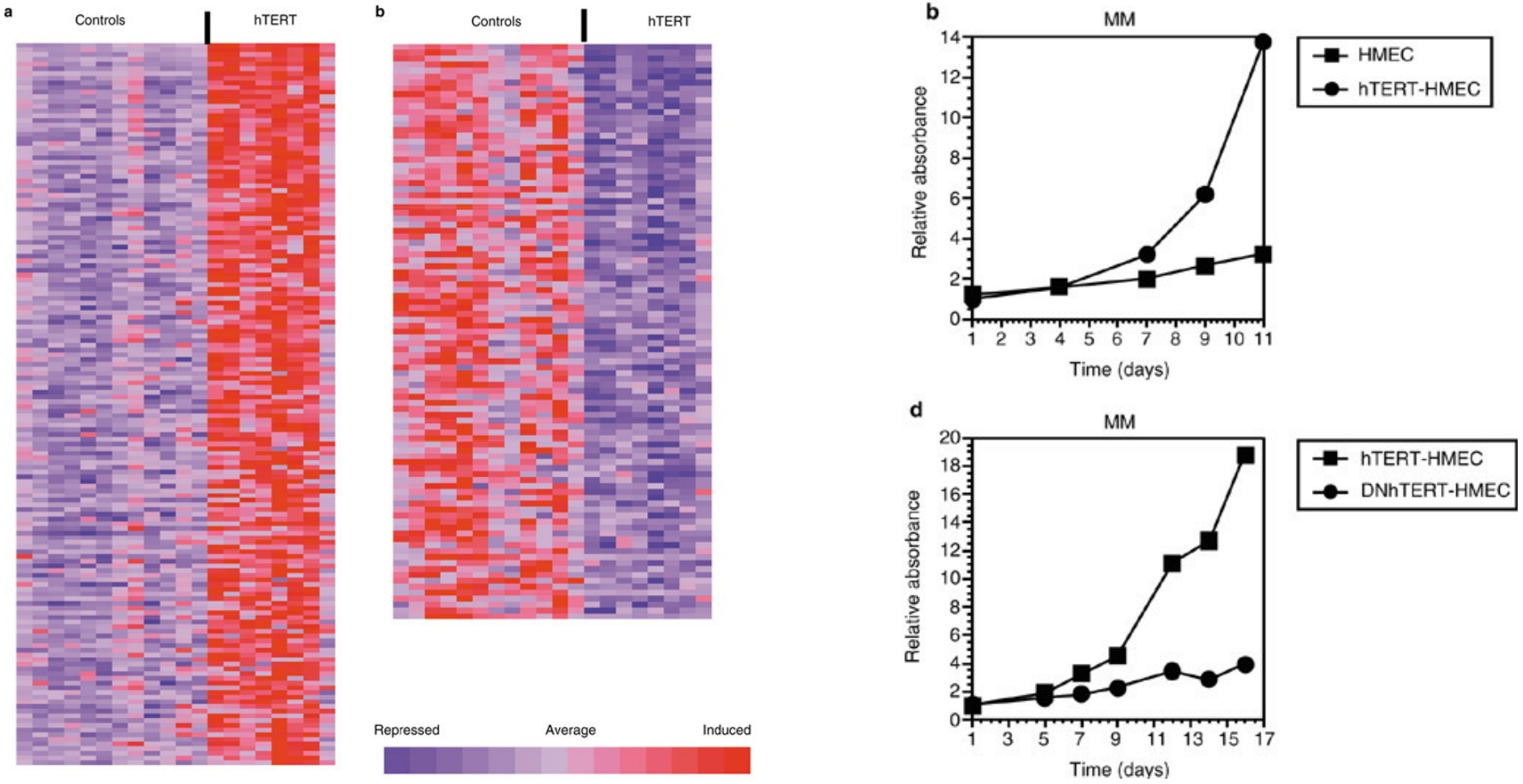
Dudognon *et al.*, (2004). Oncogene 23:7469–7474.

Rahman *et al.*, (2005). Oncogene 24:1320–1327.

Xi *et al.*, (2006). Apoptosis 11:789–798.

Del Bufalo *et al.*, (2007). Cell Death Diff 12:1429–1438.

hTERT Induces Expression of Genes that Promote Cell Proliferation

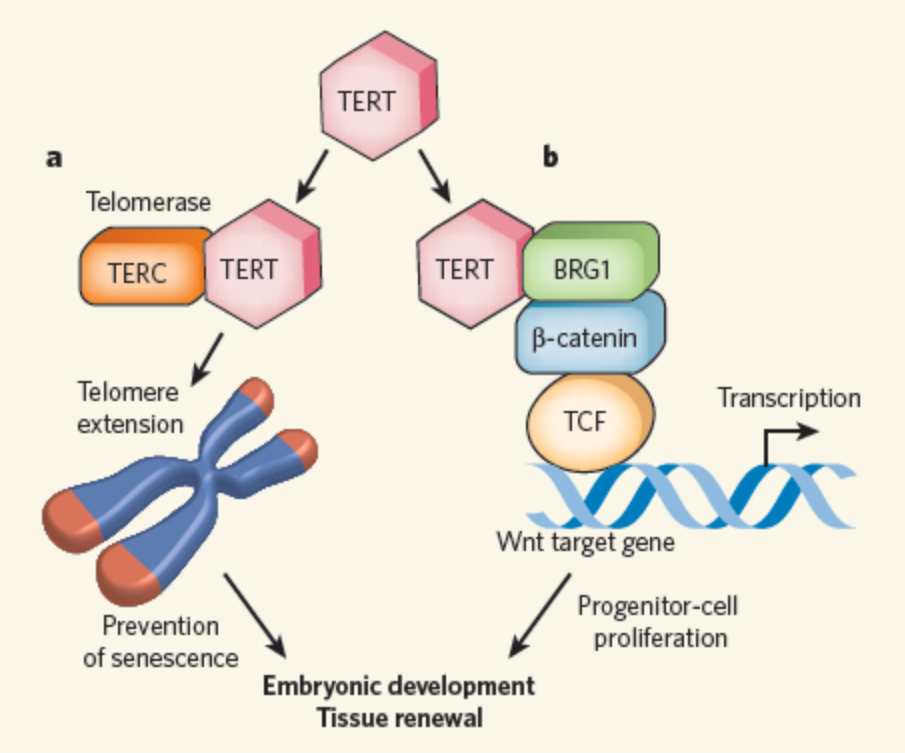


Smith *et al., (2003).* Nature Cell Biology 5, 474 - 479 (2003)

- EGFR and bFGF where among the genes upregulated by hTERT expression – these receptor tyrosine kinases have established roles in promoting cell proliferation

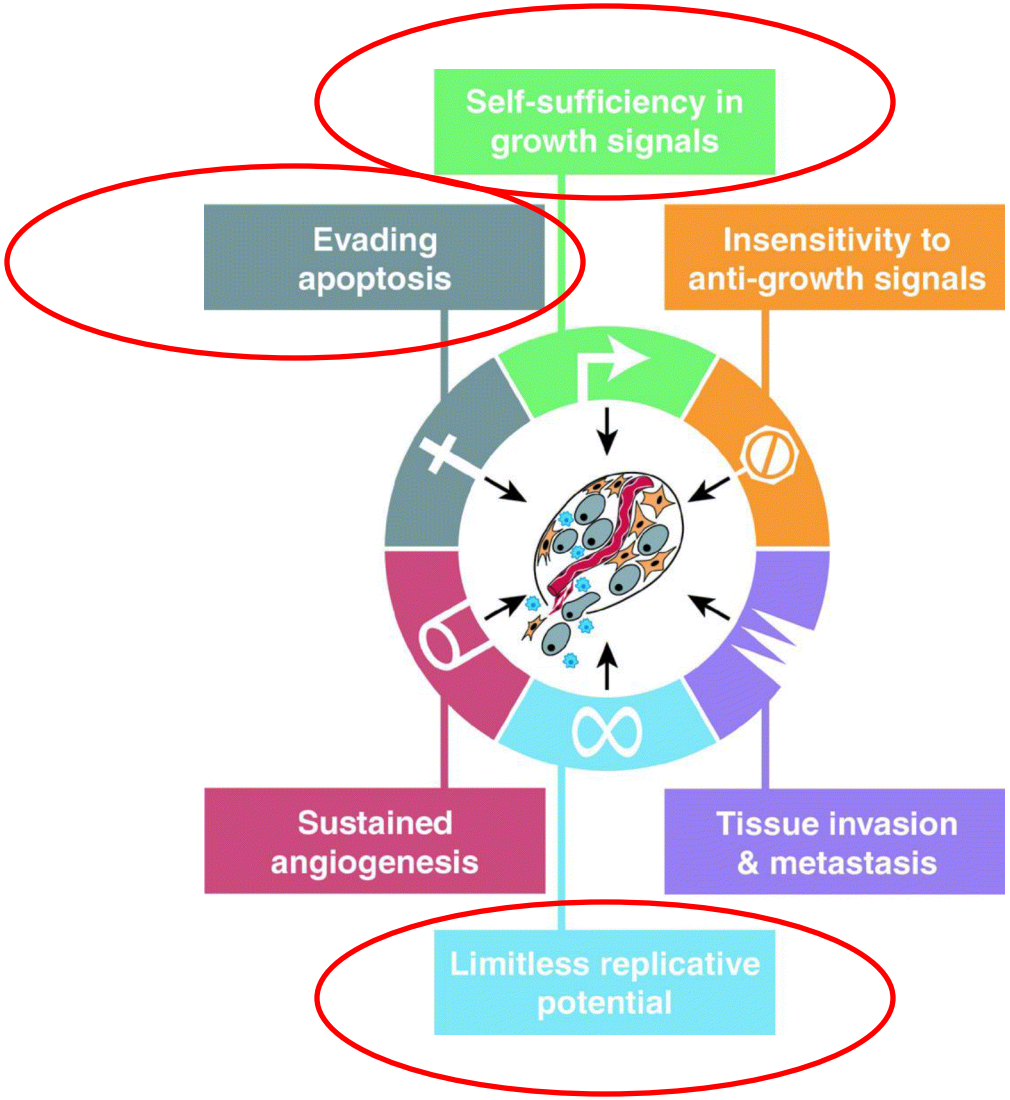
Telomerase modulates Wnt signalling by association with target gene chromatin

Jae-Il Park[1], Andrew S. Venteicher[1,2]*, Ji Yeon Hong[4]*, Jinkuk Choi[1,3], Sohee Jun[1], Marina Shkreli[1], Woody Chang[1], Zhaojing Meng[5], Peggie Cheung[1], Hong Ji[4], Margaret McLaughlin[6], Timothy D. Veenstra[5], Roel Nusse[7], Pierre D. McCrea[4] & Steven E. Artandi[1,2,3]



Millar, S.E. Nature 460, 44-45, 2009

Multiple Roles for Telomerase in Cancer



Therapeutic Opportunities

Why do Telomerase/Telomere Maintenance Represent Good Targets?

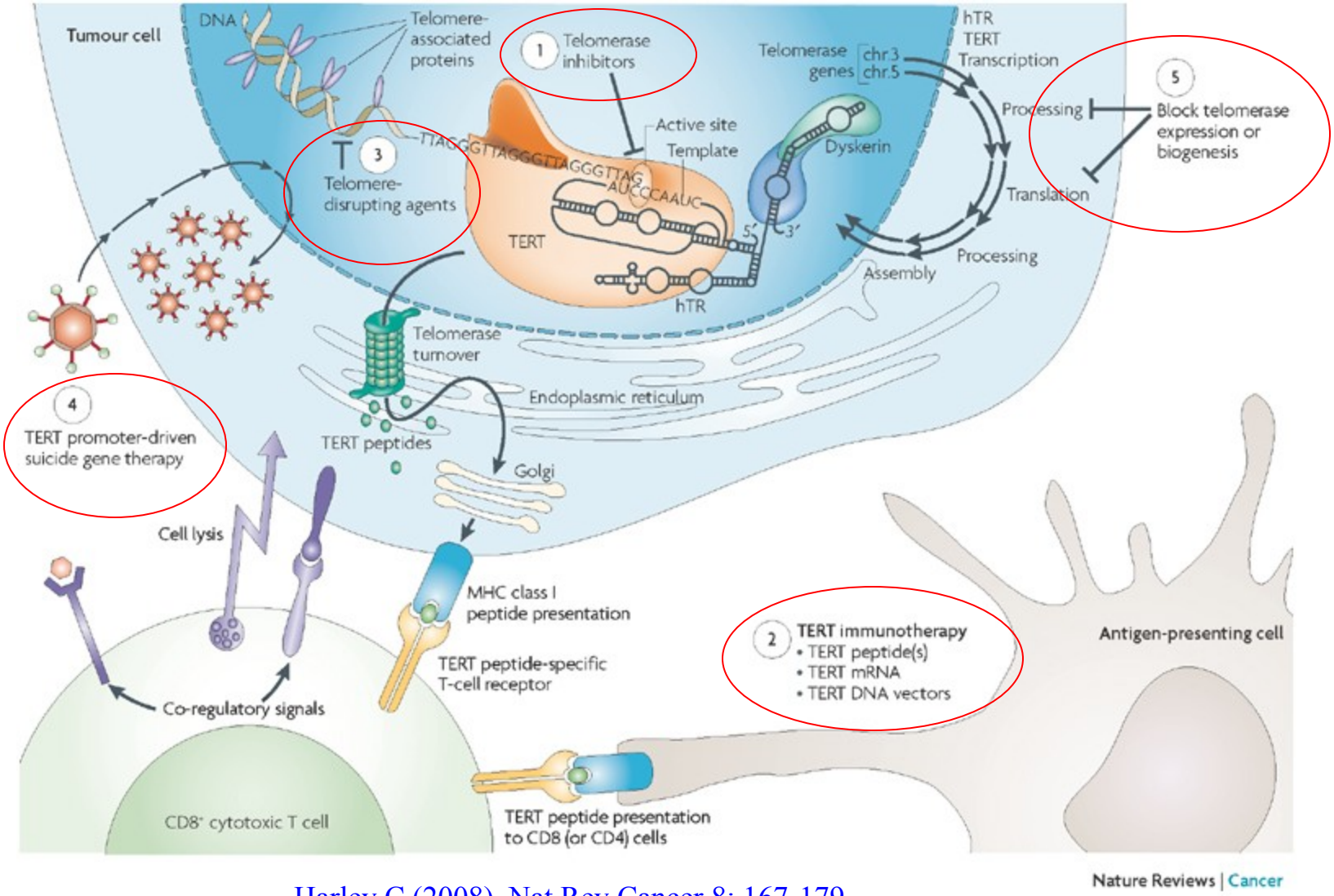
Universal Target – estimated that 80-90% of all tumors are telomerase positive

Critical Target – we have seen that Telomerase activity/Telomere maintenance is required for the transformed phenotype

Specificity – most normal human somatic cells have absent or very low telomerase whereas cancer cells upregulate telomerase expression

Cancer Stem Cells – could afford the ability to target telomerase positive cancer stem cells

Intense Interest in Telomeres/Telomerase as a Clinical Target



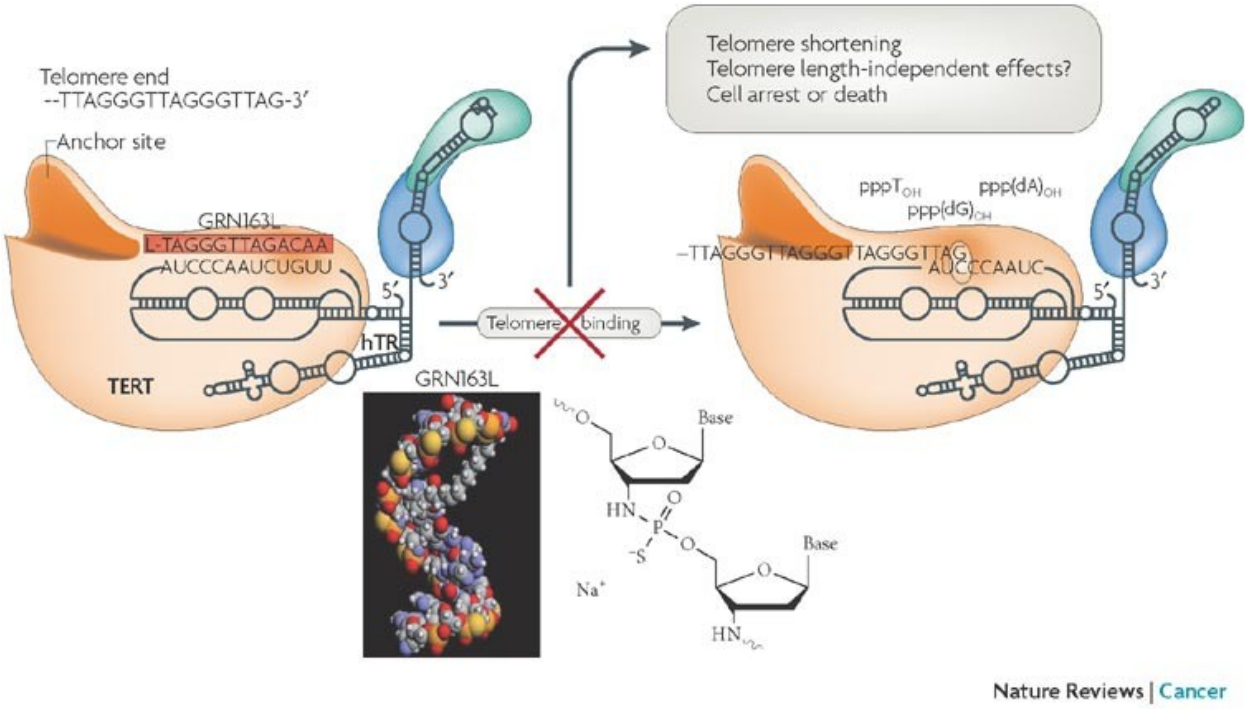
Harley C (2008). Nat Rev Cancer 8: 167-179

Strategies for Targeting Telomerase Activity
Telomerase RNA template antagonists

Telomerase RNA template antagonists:

These agents serve as competitive inhibitors that prevent the RNA component from binding to telomeres (eg. GRN163L)

in clinical trials for chronic lymphocytic leukemia (CLL), multiple myeloma, lung cancer



Harley C (2008). Nat Rev Cancer 8: 167-179

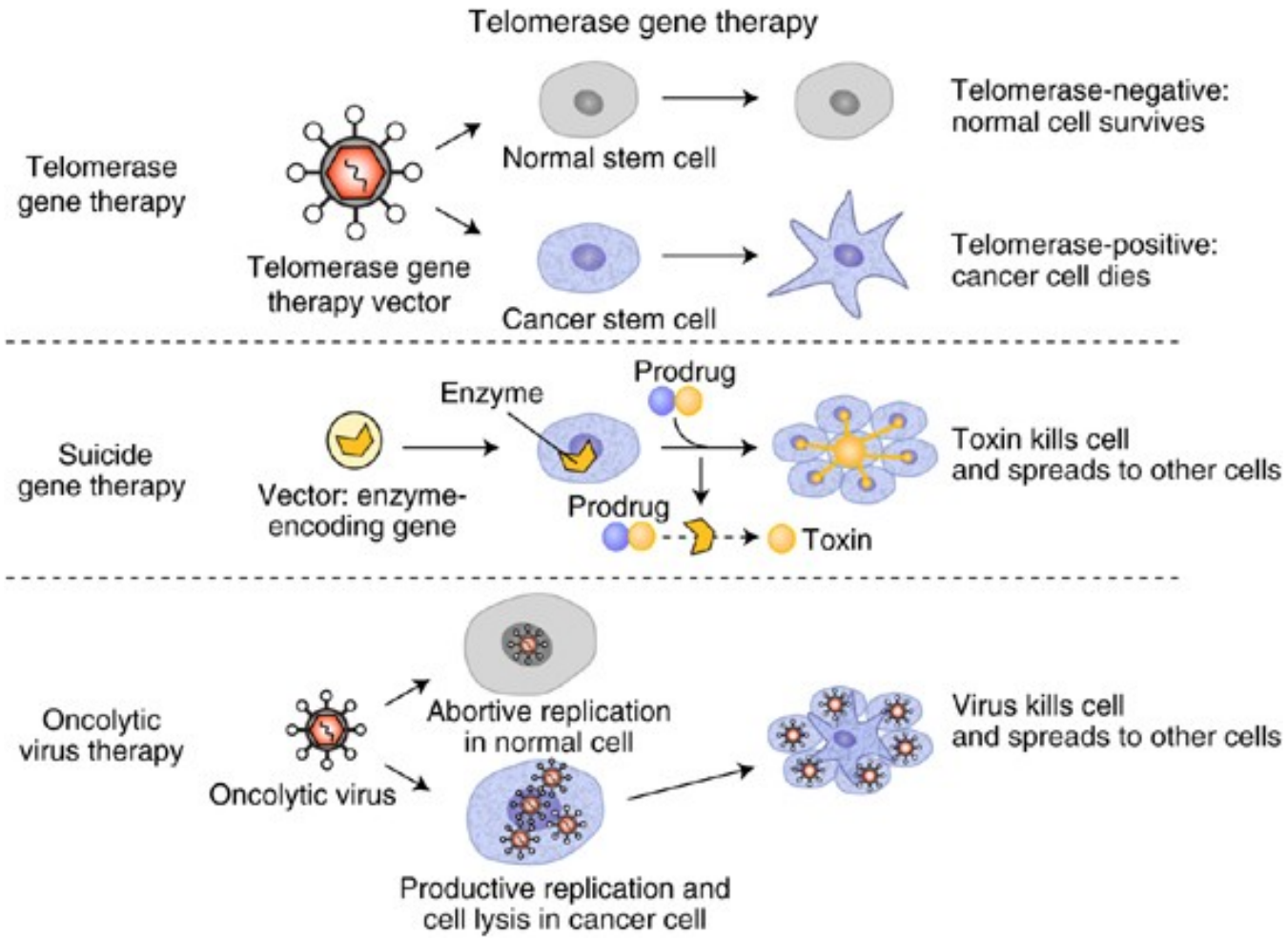
Strategies for Targeting Telomerase Activity Immunotherapy

i) Development of cytotoxic T cell (CTLs) responses against specific hTERT peptides expressed on the surface of cancer cells. Phase I/II trials underway to assess toxicity following immunization with hTERT - derived peptides

Furthest developed: GV1001 – 16mer hTERT peptide
(*NSCLC, melanoma and pancreatic cancer trials)*

ii) Stimulation of dendritic cells *ex vivo* (transfecting these cells with hTERT mRNA)
Dendritic cells process hTERT protein into multiple peptides – present multiple epitopes
Re-introduce the primed antigen presenting cells into the patient
facilitates CD8/CD4 T cell activation and anti-tumor immunity
Furthest developed: GRNVAC1 (mixture of mature autologous dendritic cells)
(*renal cancer, advanced prostate cancer, AML)*

Strategies for Targeting Telomerase Activity Gene Therapy



Delivery of anti-sense RNA, siRNA, etc. against Telomerase

Target Telomerase expression

hTERT promoter drives the expression of a suicide gene (NTR-nitroreductase) Converts an inert drug into a cytotoxic drug

hTERT promoter drives the expression of a gene that is essential for replication of an adenovirus (E1A and E1B) Eg. OBP-301

Shay and Keith. British Journal of Cancer (2008). 98: 677-683

List of ongoing human clinical trials using a variety of approaches to targeting telomerase

List of ongoing human clinical trials using a variety of approaches to targeting telomerase.

Trial identifier	Condition	Intervention	Phase
NCT00124189	Chronic lymphoproliferative diseases	Imetelstat (GRN163L)	I
NCT01242930	Multiple myeloma	Imetelstat (GRN163L)	II
NCT01256762	Locally recurrent or metastatic breast cancer	Imetelstat (GRN163L) + bevacizumab + paclitaxel	II
NCT01137968	Non-small cell lung cancer	Imetelstat (GRN163L) + bevacizumab	II
NCT01265927	Breast neoplasms	Imetelstat (GRN163L) + trastuzumab	I
NCT01243073	Essential thrombocythemia	Imetelstat (GRN163L)	II
NCT00021164	Melanoma adult solid tumor	Aldesleukin + incomplete Freund's adjuvant + telomerase 540–548 peptide vaccine	II
NCT00069940	Brain and central nervous system tumors; gastrointestinal stromal tumor; sarcoma	Telomerase 540–548 peptide vaccine + sargramostim	I
NCT00509457	Carcinoma, non-small-cell lung	GV 1001 telomerase peptide	
NCT01247623	Malignant melanoma	GV 1001 telomerase peptide + temozolomide	I, II
NCT00061035	Prostatic neoplasms	Anti-telomerase transgenic lymphocyte immunization vaccine (TLI)	I
NCT00197912	Advanced melanoma	Tumor antigen loaded autologous dendritic cells	I, II
NCT00925314	Stage III melanoma	Anti-telomerase transgenic lymphocyte immunization	II
NCT00079157	Breast cancer	Incomplete Freund's adjuvant + telomerase 540–548 peptide vaccine + sargramostim	I
NCT00425360	Pancreatic cancer	Sargramostim + telomerase peptide vaccine GV1001 + Capecitabine + gemcitabine	III
NCT00573495	Breast cancer	hTERT/survivin multi-peptide vaccine	I
NCT00197860	Advanced renal cell carcinoma	Tumor antigen loaded autologous dendritic cells	I, II
NCT00510133	Acute myelogenous leukemia	GRNVAC1	II
NCT01153113	Metastatic prostate cancer	GRNVAC1	I, II

Buseman, C.M. et al. 2012. Is telomerase a viable target in cancer? Mutation Research 730, 90-97.

Strategies for Targeting Telomeres
"Telomere Uncapping"

"Uncapping" reagents that disrupt the structure of telomere ends

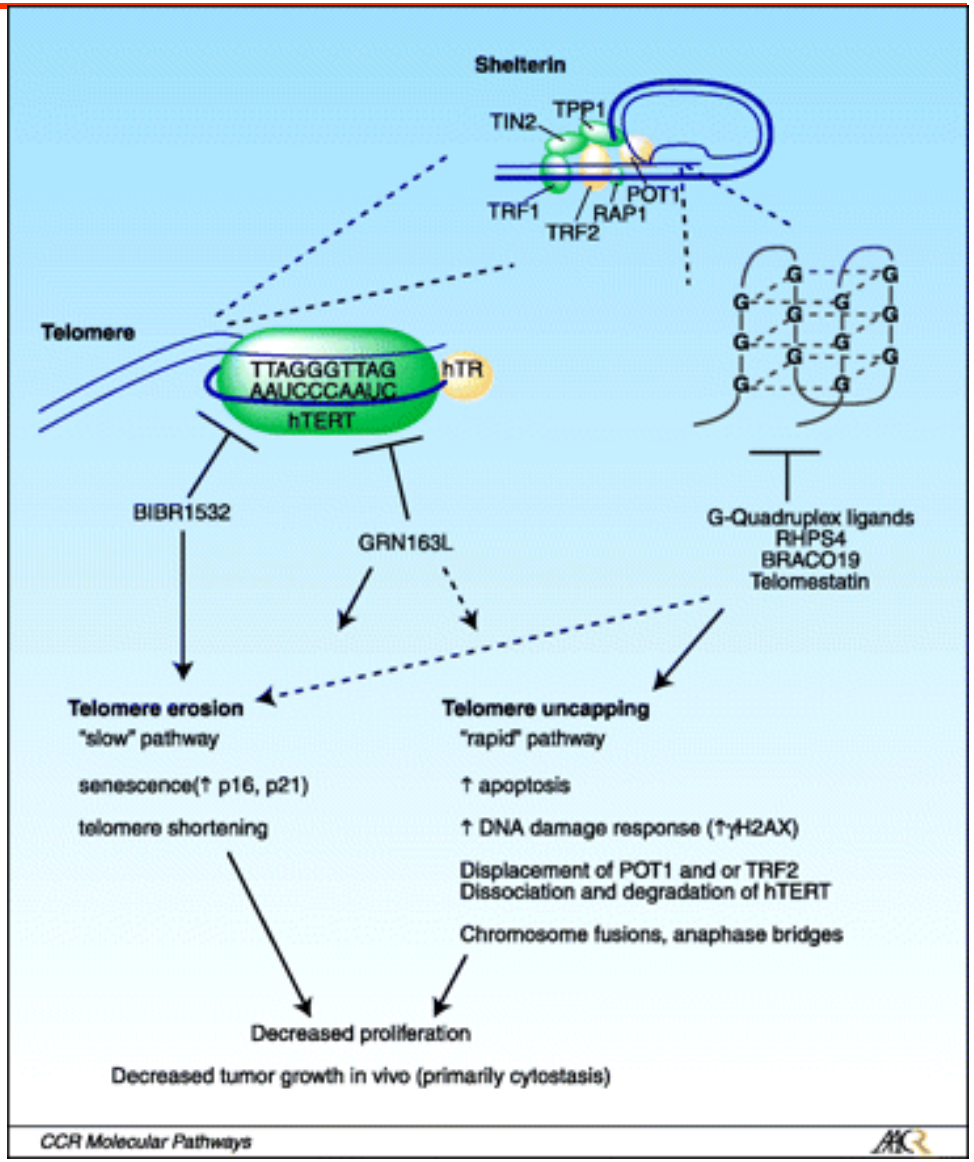
G-quadruplex reagents

BRACO19

RHPS4

Telomestatin

Advantages – do not require telomere erosion, no lag period



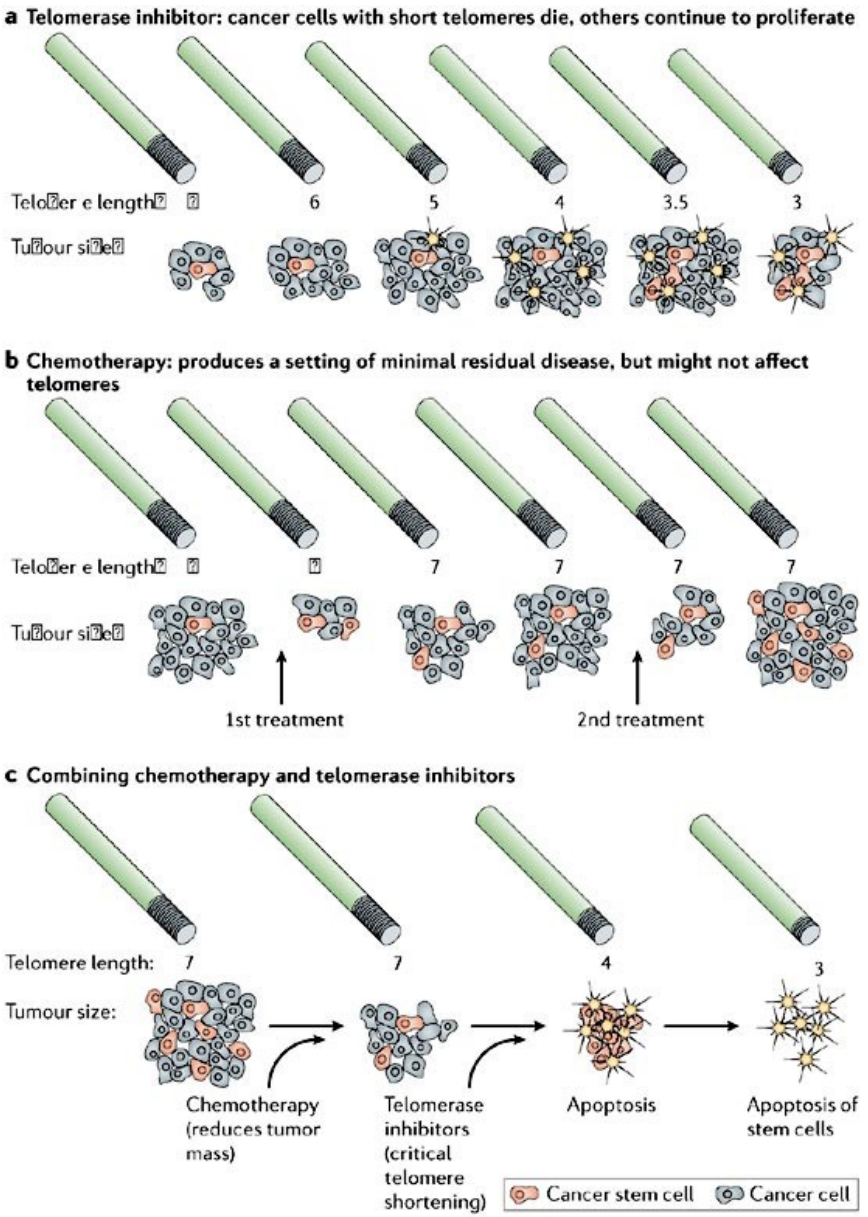
Kelland. Clin Cancer Res (2007). 13:4960-4963

Anticipating outcomes of Telomerase inhibitors on Cancer Response

Telomerase inhibition as a single agent may be ineffective – lag time allows cancer cells to adapt. Leads to recurrence.

Chemotherapy – de-bulk majority of tumor cells – not effective against tumor stem cells? Leads to recurrence.

Combination – Conventional chemotherapy kills majority of tumor cells. Telomerase inhibition can also target cancer stem cells? In this way, reduce recurrence.



Shay and Wright. Nat Rev Drug Discov. 2006 5(7):577-584.

Issues to Consider When Targeting Telomerase Function

1) Although most normal tissues are telomerase negative – certain cell types do express active telomerase (hematopoetic progenitor cells, stem cells, cells in tissues that are subject to high turnover (epidermis, mammary epithelium, colonic epithelium).

   Telomerase activity is low in these cells – and expression could be intermittent – thus, this may not be a large concern

2) Many telomerase therapies (used in isolation) will be associated with a significant lag while telomeres erode. Could be alleviated by using combination therapies.

   Strategies that result in telomere uncapping could be useful – does not require telomere erosion to occur.

Issues to Consider When Targeting Telomerase Function

3) The genotype of the tumor being treated – in the context of p53 positive tumors, inhibiting telomerase could be beneficial. *However, in, p53-/- tumors – is there a danger in the induction of further genetic instability (could this lead to a more aggressively metastatic tumor than the one initially treated?)*

   The observation that fewer genetic changes are observed going from DCIS to invasive cancer (breast) may argue against this concern

4) Drugs targeting telomerase may result in the evolution of cancer cells that are telomerase independent (drug resistance) through the upregulation of the ALT pathway

   However, some evidence exists that tumor cells using the ALT pathway are not as aggressive as telomerase positive cancer cells