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# Mechanisms of telomerase reverse transcriptase reactivation in cancer

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# *Outline*

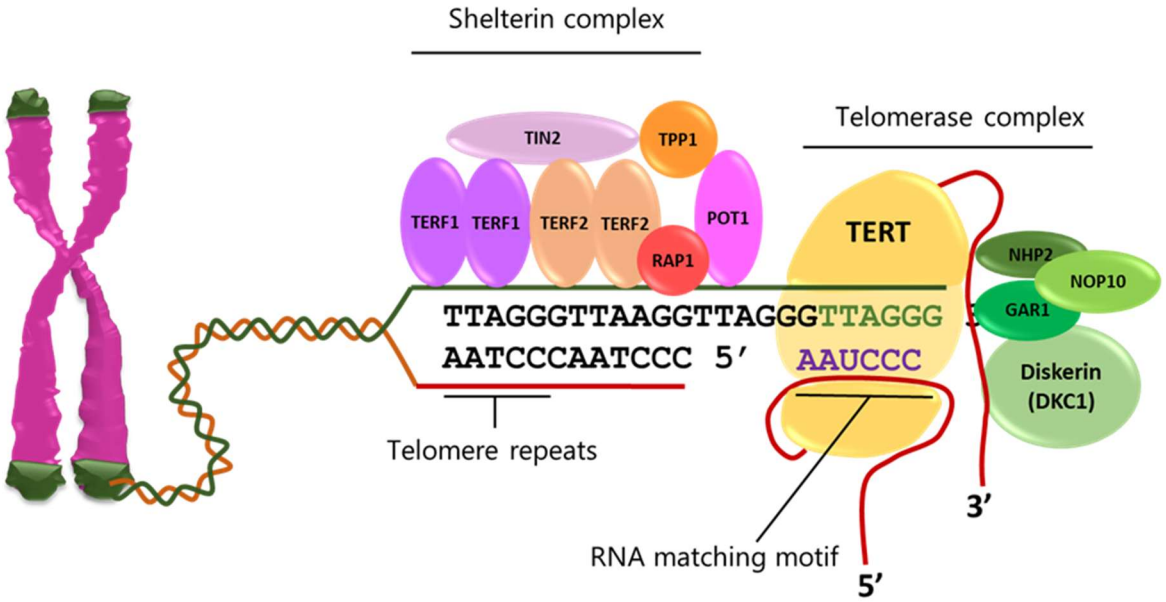
- Telomeres and telomerase
- Oncoviruses enhancing telomerase activity
- TERT promoter mutations and activation of telomerase expression
- Temporal acquisition of TERT promoter mutations in cancer
- Therapeutic opportunities

# Telomerase

- Telomerase is a ribonucleoprotein complex formed by the RNA template component (TERC) and a tetrad of proteins including dyskerin, GAR1, NHP2 and NOP10 as well as **the reverse transcriptase holoenzyme TERT**.

- Transcriptional activation of **TERT is the rate-limiting step** in the assembling of active telomerase complex. Although TERT is expressed in stem cells, it **is naturally repressed** in terminally differentiated **somatic cells.**

- Telomeres consist of **TTAGGG telomeric repeats**, forming a long region of double stranded DNA terminating in the **single stranded G-rich overhang** packaged by histones into chromatin and is bound by the sheltering complex




Tornesello et al. Front Cell Dev Biol. 2023

# Long and short telomeres

**Long telomeres**

- **Long deprotected telomeres**
- Accumulation of DNA damage
- Chromosomal fusions
- tumour formation

**Optimal lenght**

- **Optimal telomere protection**
- Telomere shorten after each replication
- Cells maintain Hayflick limit

**Short telomeres**

- **Deprotected telomeres**
- Accumulation of DNA damage
- Chromosomal fusions
- Aberrant TERT activation

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# Oncoviruses enhance telomerase activity

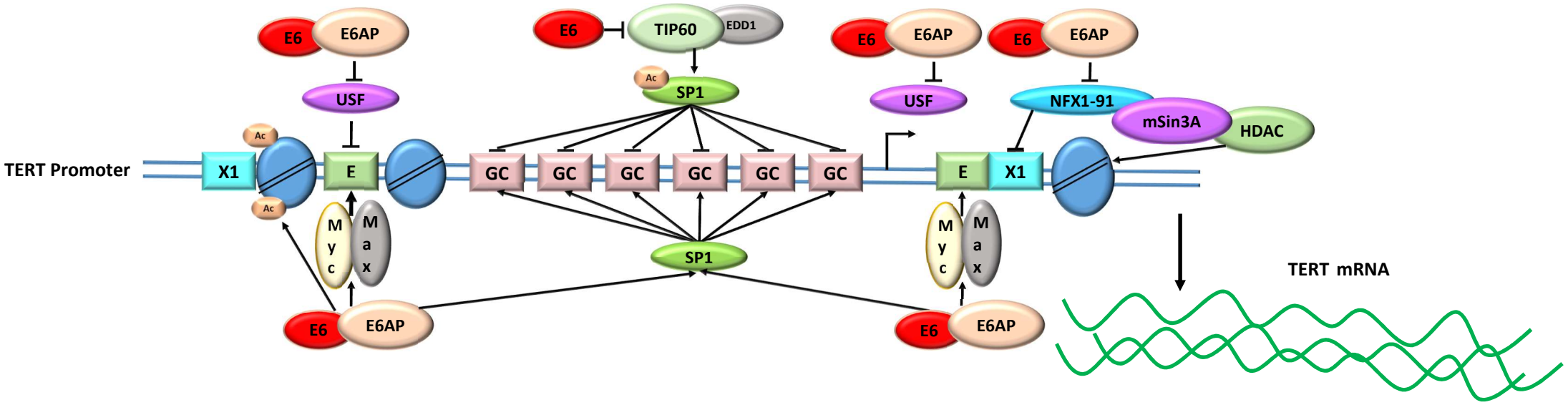
# Oncoviruses activate TERT expression

- Several **DNA and RNA oncoviruses have the ability to enhance telomerase activity** and telomere length and to contribute to the unlimited proliferation and transformation of chronically infected cells.

- Viruses, such as HBV and HPV**, can integrate their genomes nearby TERT gene locus** causing enhanced expression of TERT.

- Oncoviral proteins, such as high-risk human papillomavirus **(HPV) E6**, Epstein-Barr virus **(EBV) LMP1**, Kaposi's sarcoma-associated herpesvirus (**HHV-8) LANA**, hepatitis B virus (**HBV) HBx**, hepatitis C virus (**HCV) core protein** and human T-cell leukemia virus-1 (**HTLV-1) Tax protein**, have been demonstrated to contribute to the **transcriptional activation of the TERT gene** mainly by interacting with negative regulators of TERT transcription

# TERT activation by HPV E6




The main strategies adopted by HPV E6 to regulate telomerase activity at transcriptional level relies on the ability of **HPV E6/E6AP complex to stabilize MYC/MAX and SP1 transcription** activators, to dislocate **USF and NFX1-91 transcription repressors,** to induce the **acetylation of histone H3** and to **inhibit TIP60-mediated acetylation of SP1.**

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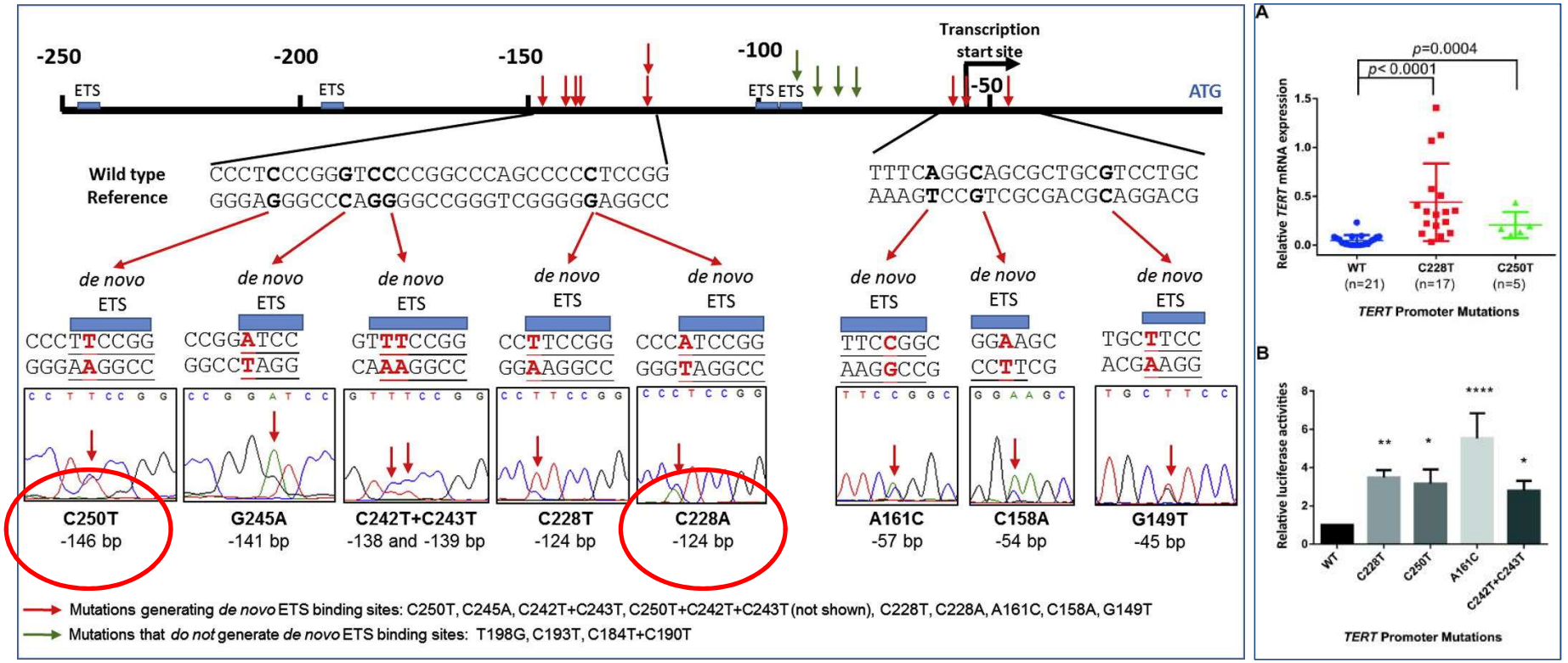
Tornesello et al. Cancers 2022

# TERT promoter mutations and telomerase expression

# Telomerase reverse transcriptase (TERT) promoter mutations

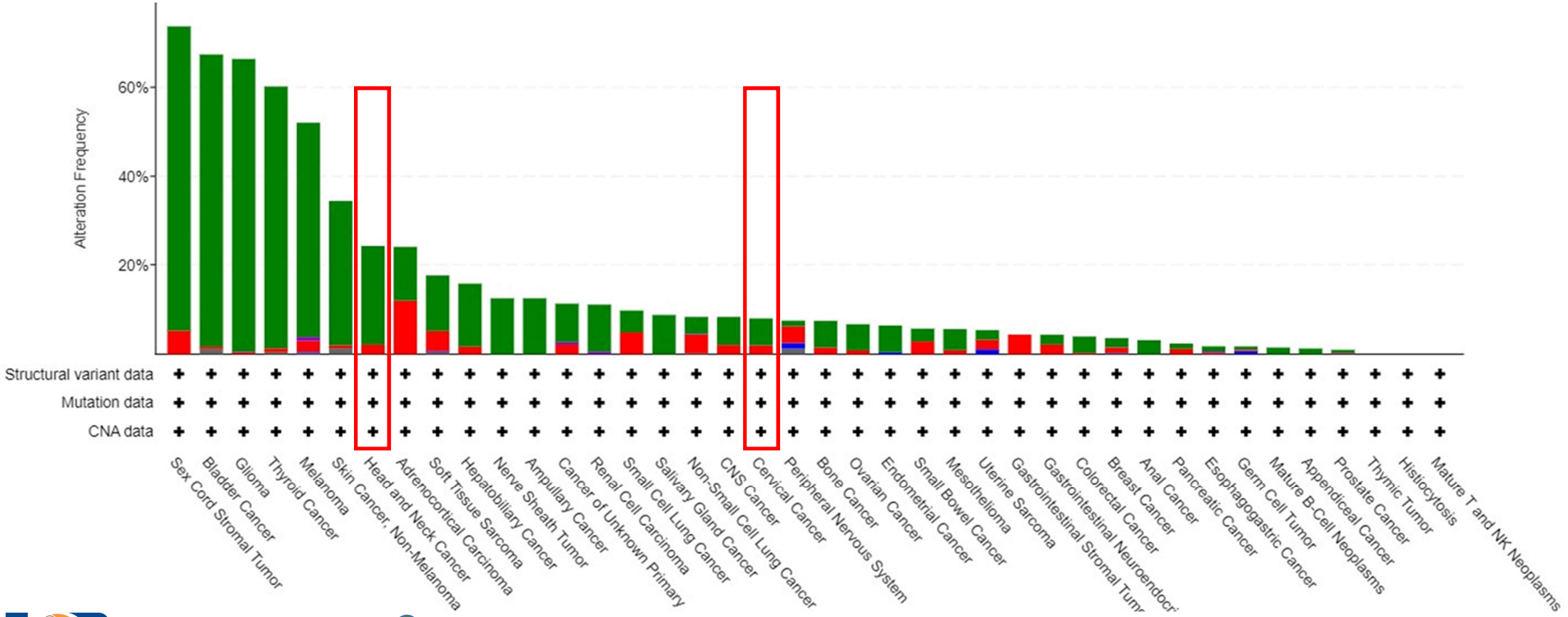






Horn et al. Science 2013, Huang et al. Science 2013

# Distribution of TERT promoter mutations by tumour type




promoter mutation

Amplification

https://www.cbioportal.org/

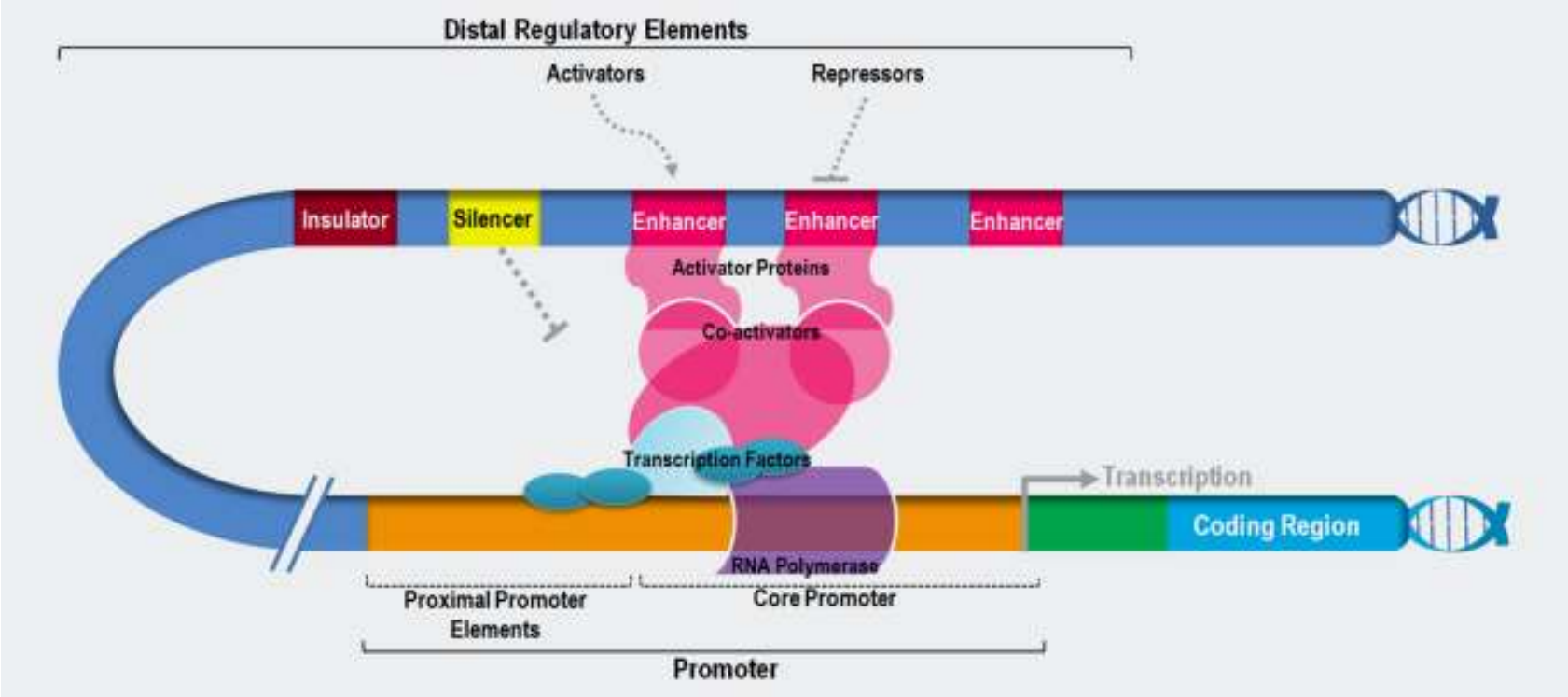
# Transcription factors and mutant TERT promoter

- TERT promoter mutations have been shown to activate telomerase expression through the **creation of *de novo* 5'-GGAA-3' binding motifs** that are recognized by transcription factors of the E-twenty-six (ETS) family, including the GA-binding proteins alpha and beta (**GABPA and GABPB**), **ETS1** and ETS Variant Transcription Factor 1 (**ETV**1)

- The transcription factor **GABPA enables the interaction of mutant TERT promoter with a distal region (T-INT1)** located **260 kb upstream TERT ATG start site** and facilitates epigenetic changes that drive TERT gene transcription

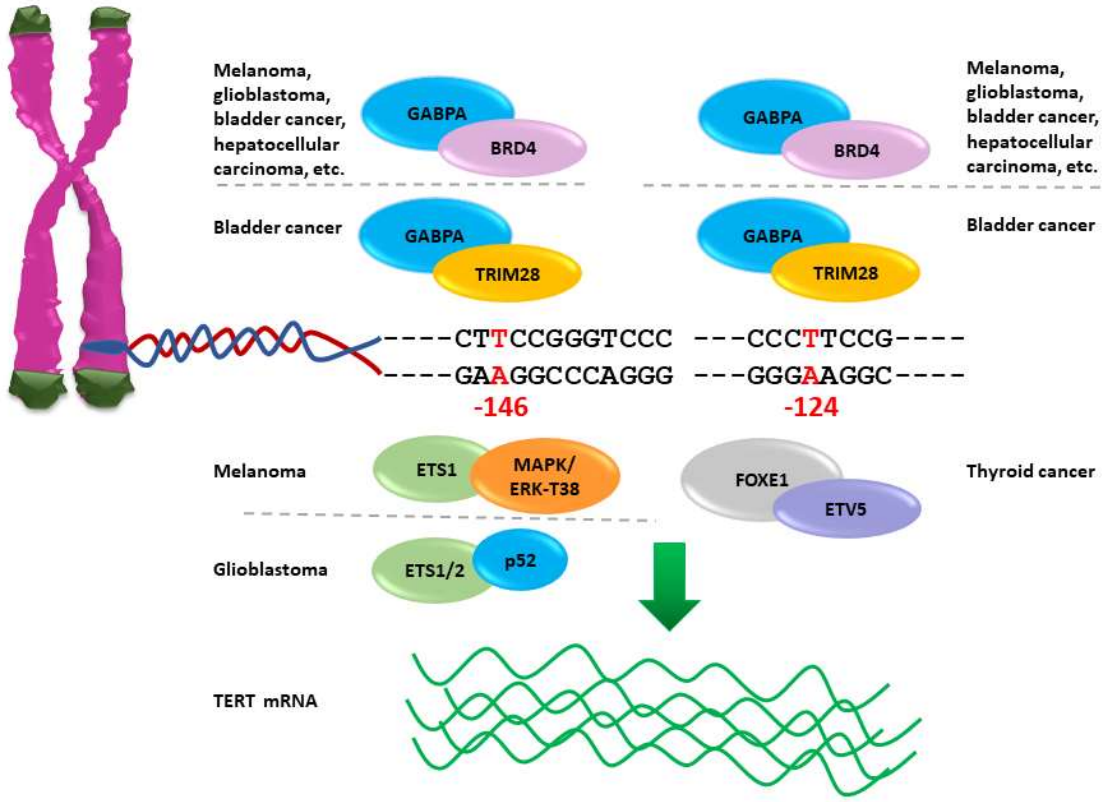
- Telomeric factors, such as TRF2, have the ability to interact with and to inhibit TERT promoter. However, mutations in **the TERT promoter cause destabilization of G-quadruplex structures and TRF2 displacement resulting** in telomerase overexpression in glioblastoma multiforme cells

# Altered TERT promoter and other regulatory elements




Kumar et al. Int J Cancer 2017

# TERT promoter mutations and novel binding sites for ETS factors

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Tornesello et al. Front Cell Dev Biol. 2023

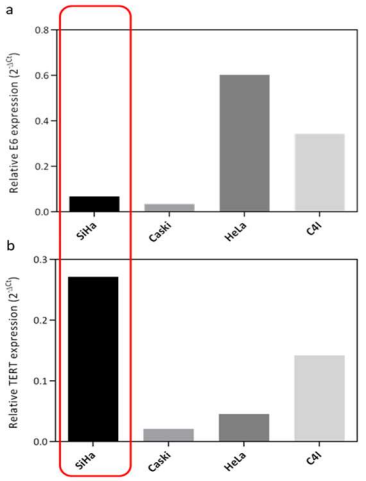
IJC International Journal of Cancer

Infectious Causes of Cancer | Full Access

# Distinct profiles of *TERT* promoter mutations and telomerase expression in head and neck cancer and cervical carcinoma

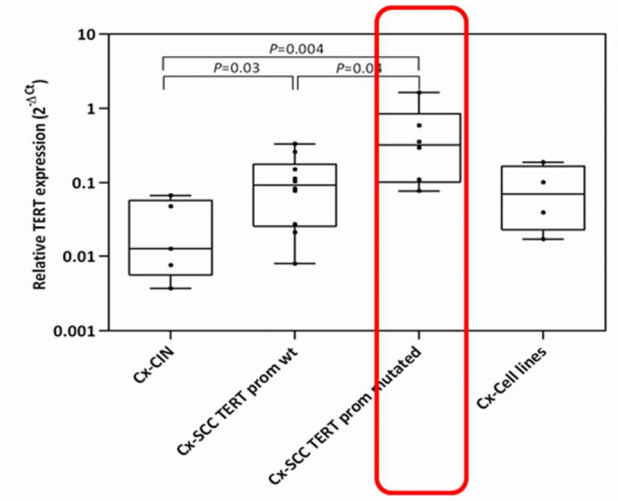
Clorinda Annunziata, Francesca Pezzuto, Stefano Greggi, Franco Ionna, Simona Losito, Gerardo Botti, Luigi Buonaguro, Franco M. Buonaguro, Maria Lina Tornesello

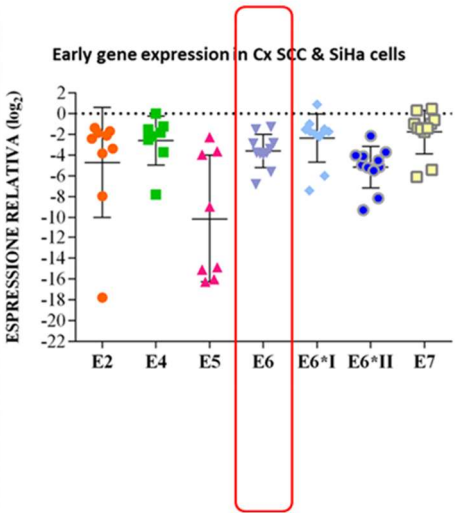
| | TERT promoter mutated cases† | % | Effect‡ |
|---|---|---|---|
| | | | |
| **Cervical SCC (n=101)** | **22** | **21.78** | |
| **Hot spot mutations** | | | |
| -124 G>A | 10 | 9.90 | ETS |
| -146 G>A | 7 | 6.93 | ETS |
| **Other mutations** | | | |
| -106 G>A | 1 | 0.99 | |
| -106 G>T | 1 | 0.99 | KLF5 |
| -111 G>A | 1 | 0.99 | |
| -115 G>A | 1 | 0.99 | ETS |
| -151 G>A | 1 | 0.99 | ETS/THAP1 |
| | | | |
| **Cervical AC (n=24)** | **1** | **4.16** | |
| **Other mutations** | | | |
| -112 G>A | 1 | 4.16 | |
| | | | |
| **CIN (n=62)** | **1** | **1.61** | |
| **Other mutations** | | | |
| -105 G>A | 1 | 1.61 | ETS |
| | | | |
| **Cervical Cell lines (n=4)**** | **1** | **25.00** | |
| | | | |
| | 1 | 25.00 | ETS |




HPV E6 levels

TERT mRNA



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IJC INTERNATIONAL JOURNAL of CANCER

# Mutations in the telomerase reverse transcriptase promoter and PIK3CA gene are common events in penile squamous cell carcinoma of Italian and Ugandan patients

Noemy Starita[1] | Francesca Pezzuto[1] | Sabrina Sarno[2] | Nunzia Simona Losito[2] | Sisto Perdonà[3] | Luigi Buonaguro[4] | Franco M. Buonaguro[1] | Maria Lina Tornesello[1]



TABLE 3 Frequency of TERTp and PIK3CA exon 9 mutations in 57 penile carcinoma samples according to the HPV status and patients provenance

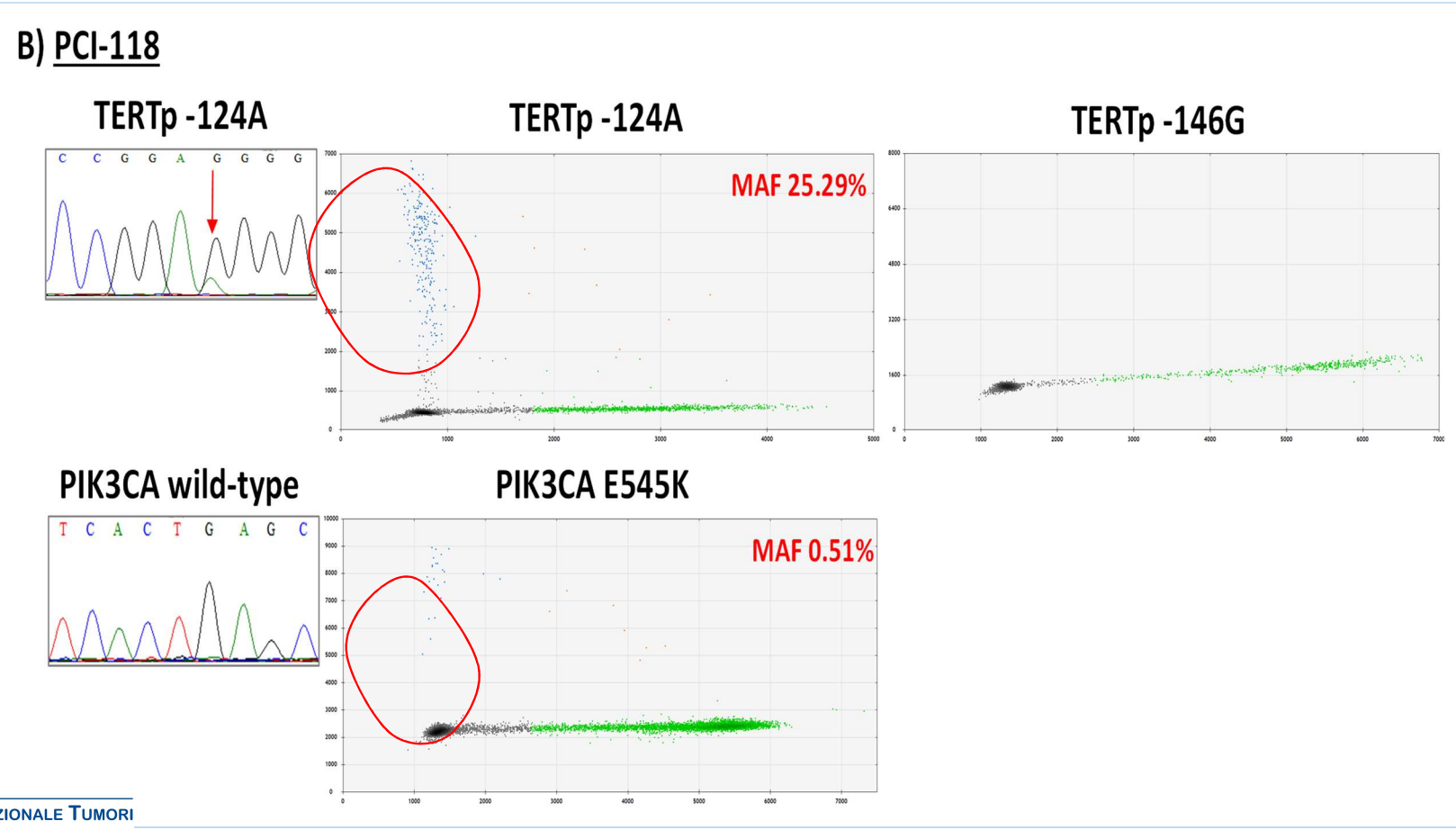
| | PIK3CA ex9 mutation (n = 15) | PIK3CA ex9 wild-type (n = 42) | |
|---|---|---|---|
| | n (%) | n (%) | *P* value |
| TERTp status | | | .5093 |
| ERTp mutated | 9 (60.0) | 21 (50.0) | |
| −124G>A | 5 (33.3) | 15 (35.7) | |
| −146G>A | 3 (20.0) | 6 (14.3) | |
| −124G>T | 1 (6.7) | 0 | |
| TERTp wild-type | 6 (40.0) | 21 (50.0) | |
| HPV status | | | .9499 |
| HPV positive | 7 (46.7) | 20 (47.6) | |
| HPV negative | 8 (53.3) | 22 (52.4) | |
| Provenance | | | .0630 |
| Italy | 14 (93.3) | 29 (69.1) | |
| Uganda | 1 (6.7) | 13 (30.9) | |



TABLE 1 HPV status, PIK3CA ex9 and TERTp mutations, detected by Sanger sequencing and ddPCR, in DNA samples extracted from penile cancer biopsies

| Sample ID[a] | HPV genotype | Tissue biopsy | PIK3CA Sanger | PIK3CA ddPCR | MAF (%) | Number of alleles screened[b] | TERTp Sanger | TERTp ddPCR | MAF (%) | Number of alleles screened[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| PCU-04 | 16, 18, 33 | Fresh | WT | WT | | 8529 | WT | WT | | 7283 |
| PCU-05 | Neg | Fresh | WT | WT | | 10 016 | WT | WT | | 9522 |
| PCU-07 | 6, 16 | Fresh | WT | WT | | 7787 | −124A | −124A | 66.79 | 5420 |
| PCU-08 | 16,18 | Fresh | WT | WT | | 10 758 | WT | WT | | 9614 |
| PCU-09 | Neg | Fresh | E545K | E545K | 25.68 | 8072 | −124A | −124A | 47.81 | 7402 |
| PCU-10 | 16 | Fresh | WT | WT | | 7771 | WT | WT | | 5488 |
| PCU-11 | 16 | Fresh | WT | WT | | 8771 | −146A | −146A | 6.85 | 7574 |
| PCU-12 | Neg | Fresh | WT | WT | | 10 350 | WT | WT | | 9541 |
| PCU-13 | Neg | Fresh | WT | WT | | 9330 | WT | WT | | 8922 |
| PCU-15 | 16 | Fresh | WT | WT | | 10 945 | WT | WT | | 9164 |
| PCU-17 | 16 | Fresh | WT | WT | | 8609 | WT | WT | | 11 681 |
| PCU-22 | 16 | Fresh | WT | WT | | 8806 | −124A | −124A | 38.98 | 6139 |
| PCU-23 | 16 | Fresh | WT | WT | | 4016 | WT | WT | | 4462 |
| PCU-24 | Neg | Fresh | WT | WT | | 10 623 | −124A | −124A | 31.05 | 8732 |
| PCI-30 | 16 | FFPE | WT | E545K | 11.19 | 1730 | WT | −146A | 10.62 | 1601 |
| PCI-31 | Neg | FFPE | E545A | WT | | 551 | −124A | −124A | 16.31 | 672 |
| PCI-32 | 16 | FFPE | WT | WT | | 643 | WT | −124A | 11.34 | 485 |
| PCI-33 | Neg | FFPE | WT | E545K | 1.13 | 678 | WT | WT | | 741 |
| PCI-34 | Neg | FFPE | WT | WT | | 421 | −146A | −146A | 42.70 | 185 |
| PCI-35 | 18 | FFPE | WT | WT | | 465 | WT | WT | | 171 |
| PCI-36 | 16 | FFPE | Q546R | WT | | 3040 | WT | WT | | 2393 |
| PCI-37 | 16 | FFPE | E545K | E545K | 18.55 | 1925 | −124A | −124A | 45.99 | 368 |
| PCI-38 | 16 | FFPE | NA | NA | | | WT | −124A | 9.09 | 294 |

# Ortologous comparison of ddPCR assays TERTp -124A, TERTp -146A and PIK3CA E545K with Sanger sequencing results.

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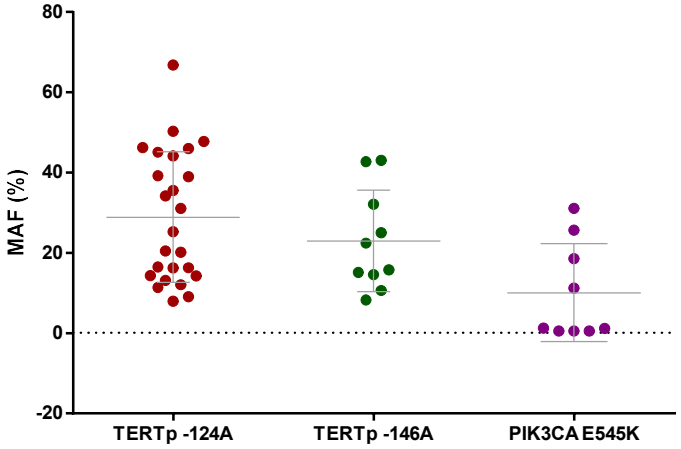
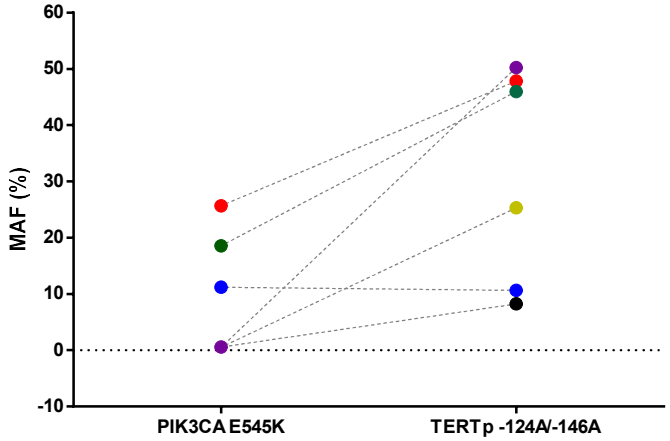
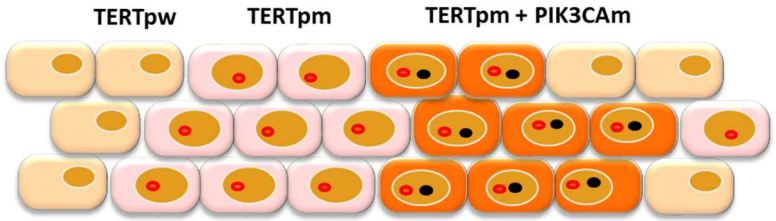
*Starita et al., Int J Cancer 2022*

# TERT promoter and PIK3CA allel mutation frequencies in penile SCC




- TERTp mutated cases showed high MAF indicating that such mutations are trunk events in penile cancer development.
- In double mutant samples, the lower PIK3CA E545K MAFs compared to TERTp -124A/-146A MAFs indicate that PIK3CA variation is a second event occurring in subclones of TERTp mutated cells

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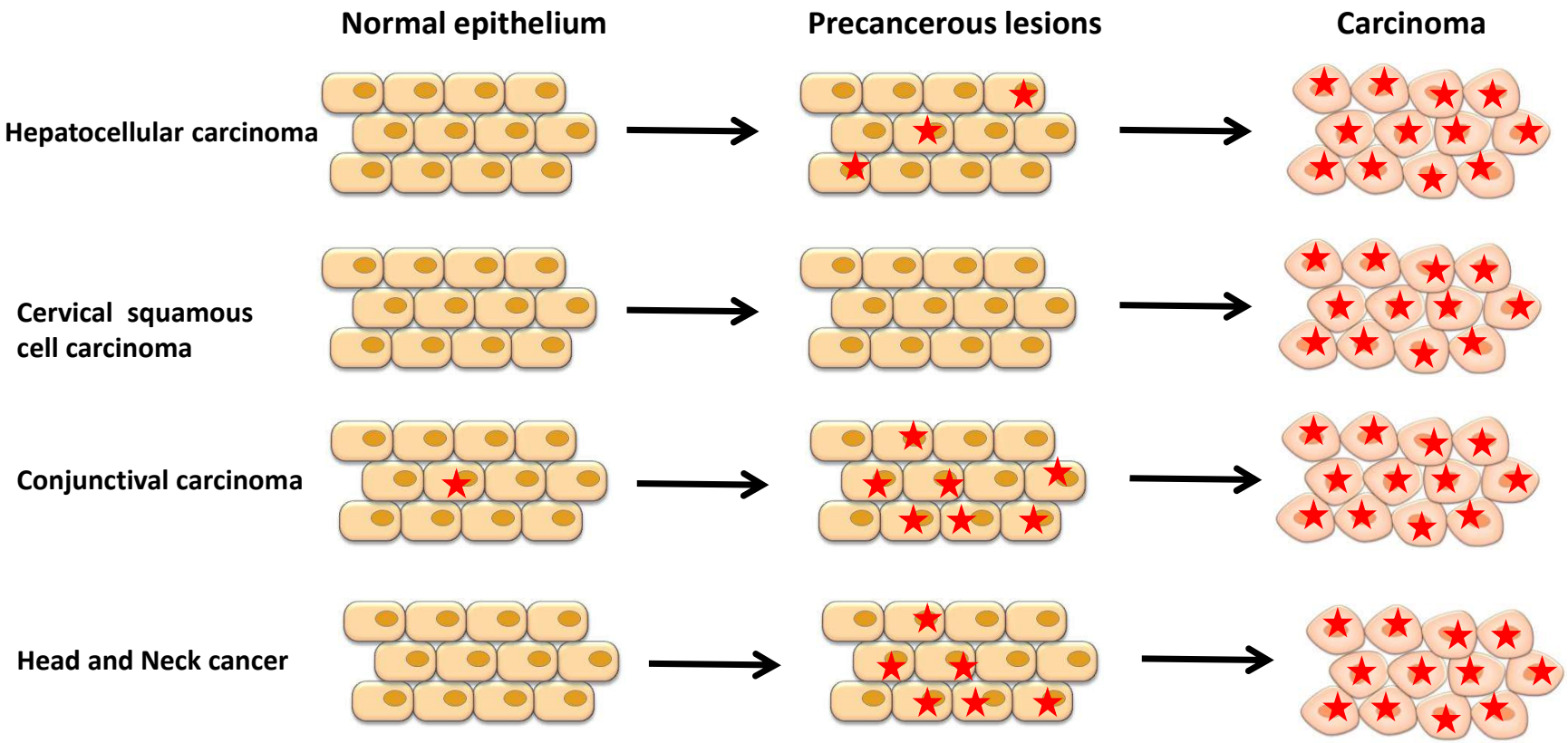
*Starita et al., Int J Cancer 2022*

# Temporal and spatial acquisition of TERT promoter mutations

- TERT promoter mutations have been defined **as early events in some tumours** and **late events** in others, consistent with the possibility that they play multiple oncogenic roles in diverse cancer types

- TERT promoter mutations are **early genetic events** in glioblastoma, melanoma, hepatocellular carcinoma, urothelial bladder cancers**, penile and conjunctival carcinoma**

# TERT promoter mutations are early or late events in Cancer

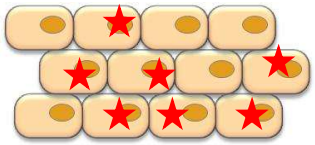


# TERT promoter mutations and HIV in conjunctiva l carcinoma

**HIV-Pos**

**Conjunctival neoplasia**

**32% TERTpm**



CIN1-2

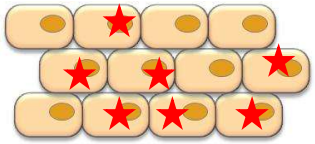
**46% TERTpm**

CIN3-SCC

**HIV-Neg**

**Conjunctival neoplasia**

**13% TERTpm**



CIN1-2

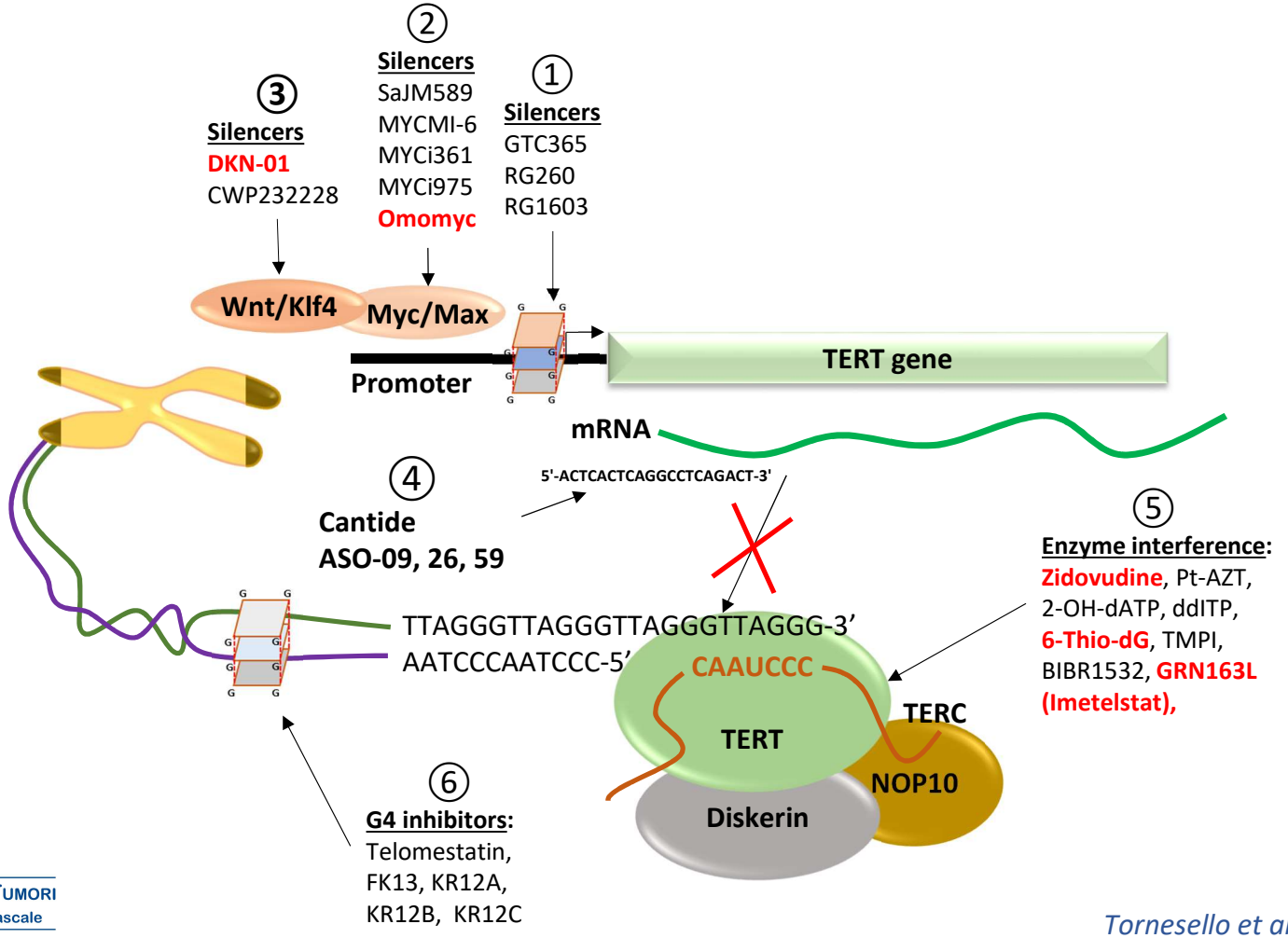
**22% TERTpm**

CIN3-SCC

*Starita et al., J Tranl Med 2018*

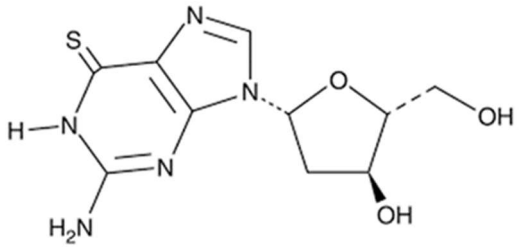
# Therapeutic strategies to target telomerase

# Therapeutic strategies to inhibit telomerase

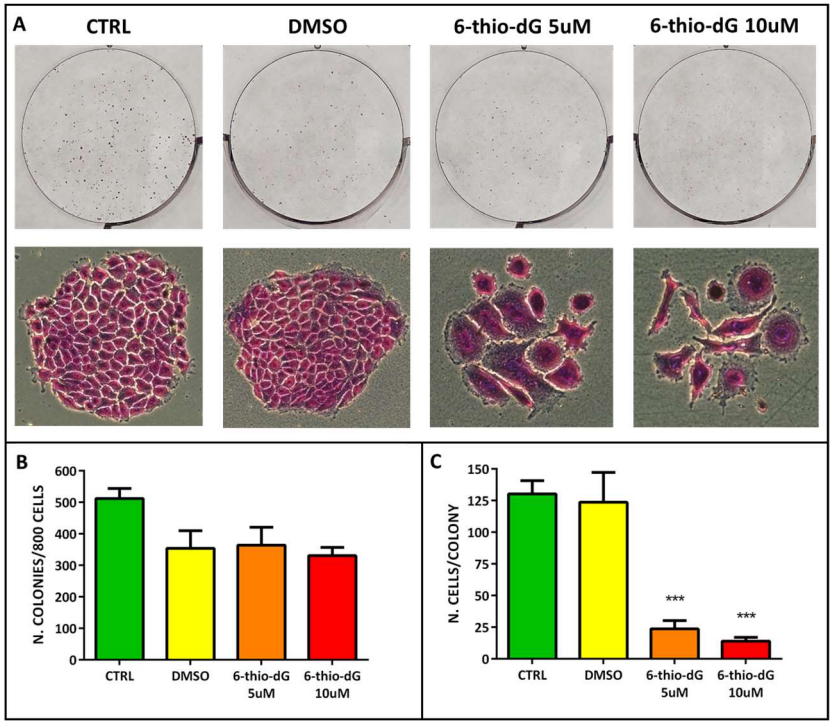



*Tornesello et al. Expert Opin Ther Targets, 2022*

# Colony formation assay with TERTpm SiHa cells




6-Thio-2'-deoxyguanosine (6-thio-dG) is a mimetic of 2'-deoxyguanosine, in which the oxygen atom of guanine is substituted with a sulfur atom. This compound is incorporated into telomeres in a telomerase-dependent manner, which causes immediate crisis in telomerase-positive cells



# The Nobel Prize in Physiology or Medicine 2009

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Elizabeth H. Blackburn

Prize share: 1/3

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Carol W. Greider

Prize share: 1/3

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Jack W. Szostak

Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2009 was awarded jointly to Elizabeth H. Blackburn, Carol W. Greider and Jack W. Szostak "for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"

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FM Buonaguro
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