Human cells possess a finite replicative life span due to a process called senescence (also termed the “Hayflick limit”), a known tumor-suppressive mechanism.

Based on your assigned readings, explain:

1) what causes replicative senescence? Describe the molecular processes involved as well as the known pathways leading to this irreversible cell cycle arrest. (25%)

•cause of replicative senescence: gradual telomere shortening due to the “end replication problem”

•ends of linear DNA molecules cannot be fully replicated because of the biochemical properties of DNA replication: necessity for a RNA primer, and synthesis in the 5’ to 3’ direction

•therefore, the very 3’ end of chromosomes cannot be fully replicated, and this leads to gradual telomere shortening at each cell division

•upon accumulation of short telomeres, occurring after 50-80 doublings depending on the cell type, a DNA damage response is induced leading to permanent cell cycle arrest in G1, termed senescence.

•the molecular pathways necessary for the induction of senescence are similar to those responding to a double strand DNA break: the MRN complex (MRE11-RAD50-NBS1), ATM and notably p53.

2) senescence is often termed “M1”, and can be followed by

another stage called “M2” (crisis). What constitutes crisis in this context? (25%)

• crisis occurs when the senescence arrest does not occur, which could happen if the cells are p53- or express an oncogene such as RAS. In this case, the cells keep dividing and accumulate short telomeres, which are deprotected and can fuse by non-homologous end joining, mediated by Ku-DNA-PK and Ligase IV. These end-to-end fusions lead to the production of dicentric chromosomes.

•crisis is characterized by a high degree of cell death, and chromosomal instability.

•the high degree of chromosomal instability is the result of breakage-fusion-bridge cycles, and are believed to result in high frequencies of translocations and broken chromosomes.

3) tumor cells are immortal in culture. Which activity confers immortality to tumor cells, allowing for the bypass of both M1 and M2?

In this case, explain what allows the bypass of M1, and of M2. (25%)

Tumor cells have undergone many mutations and aberrations, and are mostly (over 90% of the time) positive for telomerase, a dedicated reverse transcriptase which maintain telomeres in specific cell types such as stem cells and germ cells.

Telomerase adds telomeric repeat sequences to the 3’ ends of chromosomes, and thereby prevents the gradual telomere shortening which would lead to senescence. The maintenance of telomeres prevents the accumulation of short telomeres, and there is no induction of the DNA damage response, ATM and p53 which would otherwise lead to M1. Senescence is bypassed.

The maintenance of telomeres also bypasses crisis (M2) because crisis is also the result of the production of short telomeres and telomere deprotection, which do not occur upon re-expression of telomerase in tumor.

4) discuss the importance of M1 and M2 in the context of cellular transformation, and whether targeting these steps could be effective cancer therapeutic strategies. (25%)

•the bypass of M1 and M2 by telomerase (mostly) is one event leading to cellular transformation, but in itself is not sufficient for full cellular transformation. Other events required for cellular transformation include mutations in the p53 pathway, the RB pathway, and an oncogenic stimulus such as activated RAS (note: also inactivation of PP2A).

Targeting telomerase-positive cells could be a viable strategy to suppress the growth of tumor cells, especially after surgery, and to push highly proliferative cells into senescence. However stem cells would also be sensitize to such treatments. There are small compound inhibitors of telomerase which are currently used in clinical trials, and which could hopefully be used as part of a anti-tumor “cocktail” of drugs.