Targeting telomerase with the oligonucleotide GRN163L for cancer therapy

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1. Introduction

Telomeres are non-coding DNA sequences at the ends of chromosomes. Traditional DNA polymerases are unable to replicate the ends of telomeres. This incomplete replication leads to the loss of telomeric DNA with each round of cell division. Therefore, telomeres act as a "mitotic clock": progressive telomere shortening limits cellular lifespan (fig. 1).

Telomerase is a ribonucleoprotein (hTR RNA + hTERT protein) that counteracts progressive telomere shortening during cellular replication. Telomerase is not active in most human somatic cells, with the exception of proliferative stem cells.

Conversely, telomerase is reactivated in more than 85% of human cancers. The upregulation of telomerase maintains telomere length and provides cancer cells with cellular immortality (a hallmark of cancer) (fig. 1).

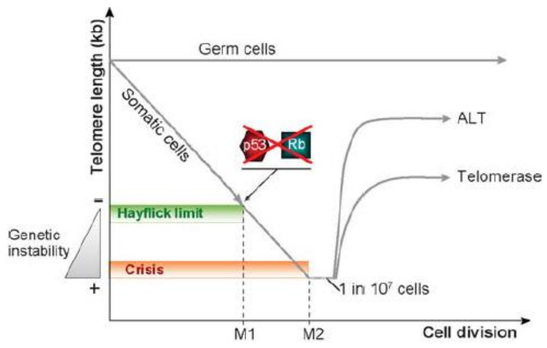

Figure 1. Telomere shortening in germ cells, somatic cells and cancer cells. In normal cells, telomere shortening induces senescence (M1). However, cells that have acquired an inactivation of cell cycle checkpoint proteins are able to keep on dividing (extended lifespan) and continue to lose telomeric sequences until they reach a crisis stage (M2), which induces apoptosis.

Cancer cells bypass senescence due to mutations in the p53/p16/Rb pathways and bypass crisis through the reactivation of telomerase or ALT pathway. Thus, cancer cells become immortal.

2. Telomerase: target for cancer therapy

Key advantages of telomerase as a target for cancer therapy:

1. Critical for cancer cell survival (fig. 2).
2. Widespread expression in 85% of human cancers (universality).
3. Not expressed in most human somatic cells (specificity).
4. Low expression in stem cells of highly proliferative tissues (low toxicity).
5. Cancer cells possess shorter telomeres than normal cells (wide therapeutic window).
6. Ability to target cancer stem cells (fig. 3).

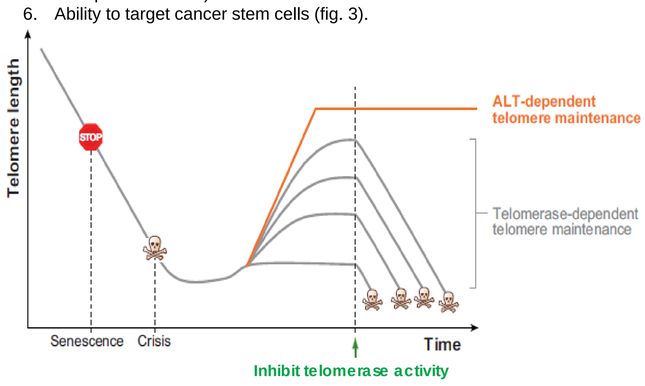

Figure 2: Inactivation of telomerase in cancer cells leads to telomere shortening and crisis, inducing apoptosis. ALT: alternative lengthening of telomeres through recombination.

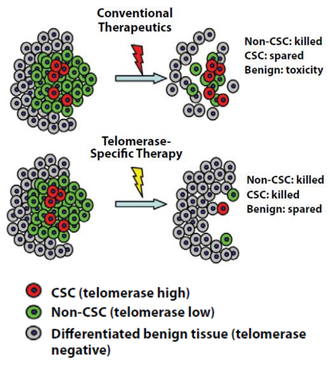

Figure 3: Telomerase-based therapies are able to target cancer stem cells (CSC). CSC are a rare population of cells within a tumor that possess self-renewal capacity and pluripotency. CSC are responsible of tumor initiation and maintenance, and often possess drug resistance mechanisms.

3. Oligonucleotides as telomerase inhibitors: GRN163L (Imetelstat®)

Oligonucleotides used for cancer therapy are N3'→ P5' phosphoramidates (DNA analogues).

GRN163L (Imetelstat) (Geron™ corporation): most successful oligonucleotide inhibitor to date.

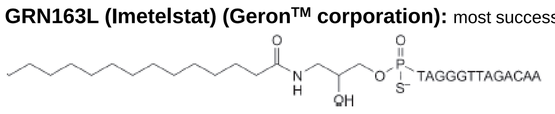

Figure 4: GRN163L chemical structure

Advantages of GRN163L for cancer therapy:

- N3' → P5' thiophosphoramidate (NPS) (fig. 4):
  - High thermodynamic stability.
  - Resistance to cellular nucleases: long half-life.
  - High target sequence specificity.
  - Low nonspecific affinity towards proteins.
  - Lack of RNaseH induction: reduces potential side effects of binding to unspecific RNAs.
- Lipid group attached to 5': lipid soluble, cell permeable, enhanced cellular uptake. (fig. 4).
- Good bioavaliability and efficient biodistribution to all major organs *in vivo.*
- Polyanionic compound: not likely to be a substrate for common mechanisms of multidrug resistance (important for targeting cancer stem cells).

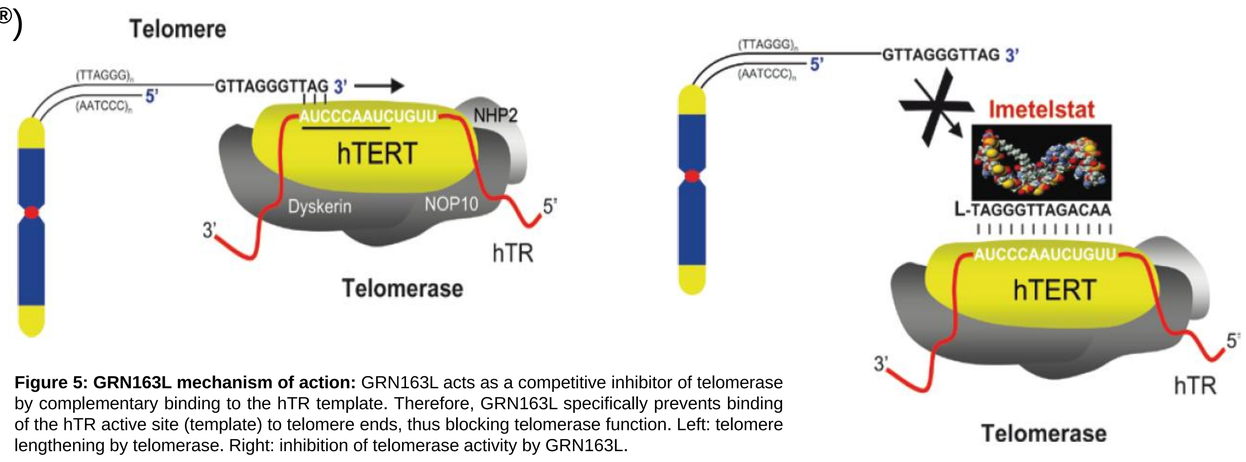

Figure 5: GRN163L mechanism of action: GRN163L acts as a competitive inhibitor of telomerase by complementary binding to the hTR template. Therefore, GRN163L specifically prevents binding of the hTR active site (template) to telomere ends, thus blocking telomerase function. Left: telomere lengthening by telomerase. Right: inhibition of telomerase activity by GRN163L.

4. GRN163L preclinical and clinical studies

4.1 Preclinical studies *in vitro* and *in vivo.*

A) Preclinical *in vitro* studies with human cancer cells

GRN163L inhibits telomerase activity

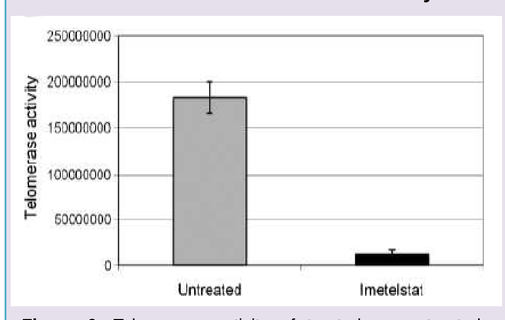

Figure 6. Telomerase activity of treated vs. untreated pancreas cancer stem cells.

GRN163L causes telomere shortening

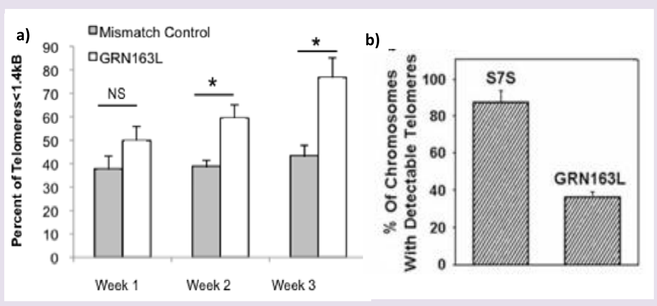

Figure 7. Human myeloma cells treated with mismatch control oligonucleotide (S7S) or GRN163L. **A)** Percentage of telomeres less than 1.4 kb (*p-value <0.05). **B)** Number of detectable telomeres.

GRN163L potentiates the effects of various radiotherapeutic and chemotherapeutic agents.

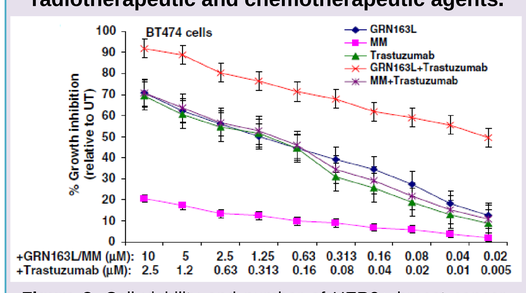

Figure 8. Cell viability and number of HER2+ breast cancer cells treated with GRN163L, mismatch control oligonucleotide (MM), Trastuzumab, or the combination of GRN163L/MM with Trastuzumab.

GRN163L decreases cell growth and colony formation capacity through induction of apoptosis.

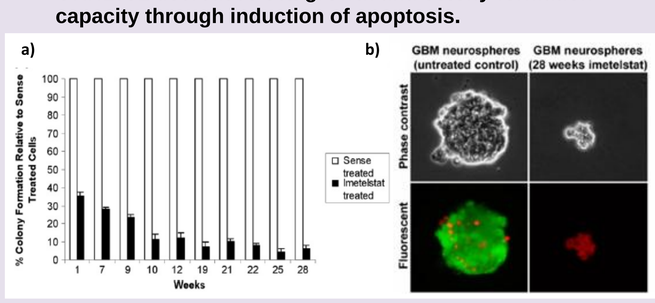

Figure 9. A) Colony formation assay of esophageal cancer cells pretreated with GRN163L compared to sense control pretreated cells. **B)** Live/dead assay on long term treated glioblastoma cancer stem cells compared to untreated controls. Green cells: live cells, red cells: necrotic cells (apoptotic).

B) Preclinical *in vivo* studies using mouse models

Treatment with GRN163L decreases tumorigenicity of cancer stem cells *in vivo*.

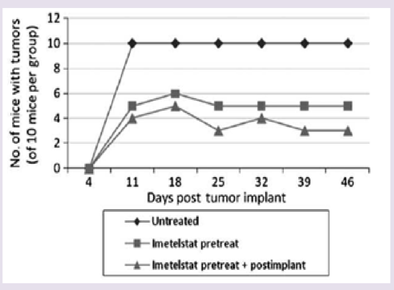

Figure 10. Tumor development in mice injected either with treated or untreated pancreatic cancer stem cells. Postimplant = secondary treatment with GRN163L *in vivo.*

GRN163L inhibits tumor growth and reduces metastases *in vivo*.

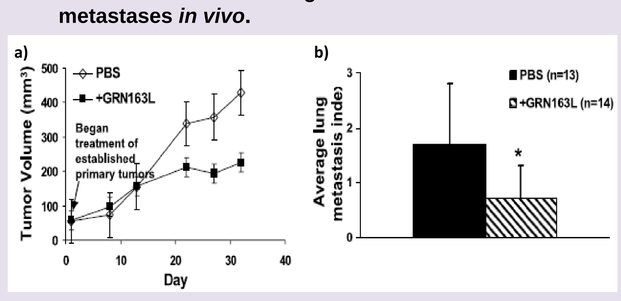

Figure 11. Human breast cancer cells (treated vs. untreated) were injected into mice. **A)** Primary tumor growth. **B)** Number and size of lung metastasis.

4.2 Clinical studies

Table 1. GRN163L phase I and I/II clinical trials for various cancers.

Phase/ Identifier	Condition	Drug Interventions
Completed		
NCT00594126 Phase I	Multiple Myeloma	GRN163L
NCT00510445 Phase I	Non-small cell lung cancer	GRN163L, Paclitaxel, Carboplatin
NCT00718601 Phase I	Multiple Myeloma	GRN163L, Bortezomib, Dexamethasone
NCT00732056 PhaseI/II	Breast cancer (recurrent or metastasic)	GRN163L, Paclitaxel, Bevacizumab
NCT01256762 Phase II	Breast cancer (recurrent or metastasic)	GRN163L, Paclitaxel with or without Bevacizumab
Ongoing		
NCT00124189 Phase I	Chronic Lymphoprolife-rative diseases	GRN163L
NCT00310895 Phase I	Solid tumor malignancies	GRN163L
NCT01137968 Phase II	Non-small cell lung cancer	GRN163L, Bevacizumab
NCT01243073 Phase II	Essential thrombocythemia	GRN163L, standard of care
NCT01265927 Phase I	Her2+ breast cancer	GRN163L, trastuzumab
Recruiting		
NCT01242930 Phase II	Multiple myeloma	GRN163L, standard of care
NCT01273090 Phase I	Solid tumors, lymphoma	GRN163L

Outcomes:

- Good safety profiles and excellent pharmacokinetics and biodistribution properties.
- Maximum tolerated dose (MTD) = 9.4 mg/kg
- Toxicities: Increased coagulation time (aPTT), neutropenia and thrombocytopenia at doses higher than MTD.

5. Conclusion and future prospects

Conclusion: Preclinical studies and clinical trials demonstrate that GRN163L is an effective and promising drug for human cancer therapy. Anyway, the following issues need to be furher investigated:

What are the main safety concerns regarding telomerase inhibition therapies?

As normal stem cells transiently express telomerase, one major concern associated with the use of telomerase inhibitors is the potential decline of regenerative capacity in stem cells (not seen to date).

Will tumor cells evolve escape mechanisms to telomerase based therapies?

Pre-existing or new refractory cancer cells are likely to be found regardless of the therapeutic approach. Therefore, a significant consideration is the potential for selecting for the alternative lengthening of telomeres (ALT) pathway.

Which are the drawbacks when using telomerase inhibitors such as GRN163L?

The phenotypic lag: treatment with telomerase inhibitors may require many rounds of cell division until telomeres become critically short and apoptosis is induced.

Which are the most suitable applications for telomerase inhibitor drugs like GRN163L?

Using GRN163L in combination with conventional chemotherapy and radiotherapy treatment should lead to a more durable response and decreased disease recurrence (fig. 12).

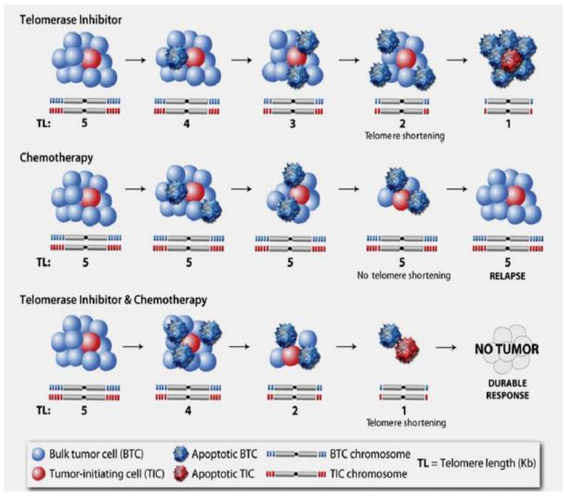

Figure 12. Synergistic effects are observed when combining GRN163L with traditional chemotherapeutic drugs.

Bibliography: 1. Röth A *et al*. Recent Results Cancer Res. 2010;184:221-34.Review, 2. Ruden M, Puri N. Cancer Treat Rev. 2012 Jul 26.