Breast Cancer Research and Treatment (2006) 96: 73–81
DOI 10.1007/s10549-005-9043-5
© Springer 2005

Effects of a novel telomerase inhibitor, GRN163L, in human breast cancer

Ginelle C. Gellert[1], Z. Gunnur Dikmen[1], Woodring E. Wright[1], Sergei Gryaznov[2], and Jerry W. Shay[1]
[1]*Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, Texas, USA;* [2]*Geron Corporation, Menlo Park, CA, USA*

Key words: breast cancer, oligonucleotide, telomerase inhibitor, therapeutics

Summary

Telomerase activity is undetectable in most normal tissues but the vast majorities of cancers express active telomerase. Therefore, telomerase serves as an attractive target for the treatment of cancers. GRN163L is a lipid-modified oligonucleotide N3′ → P5′ thio-phosphoramidate complementary to the RNA template region of human telomerase. The anti-telomerase activity of GRN163L was evaluated using MDA-MB-231 and MDA-MB-435 human breast adenocarcinoma cell lines. Twice weekly administration of GRN163L resulted in the inhibition of telomerase activity and progressive telomere shortening. Cells treated with GRN163L did not demonstrate decreased cell proliferation for up to 2 weeks. However, after additional treatment, cell proliferation gradually decreased in GRN163L-treated cells compared to untreated or mismatch control oligoncleotide treated cells. Furthermore, anti-tumorigenic effects were seen in cells treated with GRN163L, as cells lose their ability to form colonies in soft agar and were unable to form colonies in the clonal efficiency assay upon incubation with GRN163L. Moreover, breast cancer cells that were treated with GRN163L for only 1 week prior to plating in invasion chambers, and when bulk telomere are still long, exhibit significantly diminished invasive potential. These results reveal critical information regarding the effectiveness of GRN163L as a potential therapeutic agent for the treatment of human breast cancer.

Introduction

Telomeres are highly specialized structures at the ends of chromosomes consisting of multiple tandem copies of TTAGGG repeats and have a functional role of protecting chromosomal ends [1]. In most human somatic cells, shortening of telomeres occurs due to incomplete DNA replication of the lagging strand and other end processing events [2]. Progressive shortening of the telomere with each cell division ultimately results in cellular senescence when a single or a few telomeres are recognized as uncapped DNA or as double strand DNA breaks. Therefore, human primary cells have a limited capacity to divide [3]. Nonetheless, telomere shortening and subsequent cellular senescence can be overcome by the expression of the enzyme telomerase. Human telomerase is a ribonucleoprotein complex containing a reverse transcriptase catalytic subunit (hTERT) and a RNA (hTR or hTERC) component. Part of hTR is used as a template for the synthesis of TTAGGG DNA repeats [4]. While telomerase activity is not detected at high levels in the majority of somatic cells, some activity has been detected in a subset of proliferative cells, such as stem and germline cells [5]. Studies have shown that expression of the hTERT component of telomerase in primary somatic cells, including human mammary epithelial cells (HMECs), is sufficient to by-pass replicative senescence and lead to cellular immortalization [6–8]. These cells are capable of indefinite cell division, but they are not oncogenic and retain normal cell morphology and a diploid chromosome constellation [9].

In almost all human cancers, including breast cancer [10], telomerase is expressed [11–13], as it is probably needed for sustained long-term tumor growth [6,13]. Telomere length, however, is typically shorter in tumor cells when compared to adjacent non-cancerous cells [9]. Moreover, normal breast tissue and most specimens taken from patients with fibrocystic disease or from patients with benign breast lesions lack telomerase activity [14–16]. Telomerase activity is clearly associated with the acquisition of a more malignant phenotype in breast cancer, and there is a positive correlation between hTERT mRNA expression levels and the aggressiveness of breast tumors [10,17]. Moreover, a link between telomerase activity and breast tumor size and its stage has been documented [18]. Consequently, it is well recognized that telomerase serves as an attractive anticancer target. Therefore, inhibition of telomerase as a treatment option for breast cancer patients would be expected to specifically target cancer cells, while limiting

toxicity in non-cancerous cells. Current treatments targeting telomerase include telomerase template antagonists, hTERT promoter replication competent adenoviral approaches (oncolytic virus therapy), dominant negative mutant hTERT (gene therapy), immunotherapy (vaccines), and reverse transcriptase inhibitors [19]. Of these approaches, the RNA template region (hTR) presents an accessible target for the use of oligodeoxynucleotide-based telomerase inhibitor strategies [19].

Previous *in vitro* studies have shown that the application of 2′-methoxy oligonucleotides targeted to hTR result in inhibition of telomerase, progressive telomere shortening and cell death of cancer cells [20]. Also, thio-phosphoramidate oligonucleotide, GRN163L [developed in collaboration with Geron Corporation (Menlo Park, CA)], addressed the functional RNA component of the telomerase holoenzyme. GRN163L is not a 'classic' oligonucleotide anti-sense inhibitor-based approach that targets mRNA. Instead, GRN163L acts as a competitive inhibitor of the enzymes interfering with its ability to bind telomeres. The GRN163L compound is water soluble, resistant to nucleases, and demonstrates high thermal stability of duplexes formed with the complementary RNA strands [21–23]. GRN163L has a 5′-palmitate group which facilitates cellular uptake without additional lipid carriers or other cellular uptake enhancers.

Herein, we show that GRN163L is an effective telomerase inhibitor in breast cancer-derived cell lines. Treatment with GRN163L resulted in growth inhibition, reduction of colony formation in soft agar and reduced invasion through Matrigel™, even after only a few treatments (1–2 weeks). Therefore, these results provide critical pre-clinical data that may permit the use of GRN163L for *in vivo* experiments and for the potential treatment of patients with breast cancer.

Materials and methods

Cell lines

The MDA-MB-231 breast adenocarcinoma cell line, BJ human fibroblasts and HT1080 human fibrosarcoma cells were grown in a 4:1 mixture of Dulbecco's modified Eagle's medium and medium 199 supplemented with 10% cosmic calf serum (HyClone) and 50 mg of gentamycin (Sigma). The MDA-MB-435 human breast adenocarcinoma cancer cells were grown in RPMI with 10% FBS.

Telomerase activity assay

Telomerase activity was measured using a TRAP-eze kit (Intergen). Briefly, 1×10^5 cells were pelleted and lysed for 30 min in ice-cold NP-40 lysis buffer (10 mM Tris–HCl pH 8.0, 1.0 mM $MgCl_2$, 1 mM EDTA, 1% NP-40, 0.25 mM sodium deoxycholate, 10% glycerol, 150 mM NaCl, 5 mM β-mercaptoethanol). Samples were mixed with TRAP-eze kit reagents according to manufacturer's instructions, and the telomerase extension products were amplified using PCR (94 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s; 30 cycles) in the presence of a Cy5-labeled TS primer. Samples were resolved on a 10% polyacrylamide gel and scanned using a STORM 860 PhosphorImager scanner system (Molecular Dynamics).

Telomere length

To measure telomere length, 1×10^6 cells were collected and lysed and digested in lysis buffer (100 mM NaCl, 100 mM EDTA pH 8.0, 10 mM Tris pH 8.0, 1% Triton X-100 and 2 mg/ml proteinase K) overnight at 55 °C. Following the digestion, proteinase K was inactivated at 70 °C for 30 min, and the samples were dialyzed in TE buffer (10 mM Tris–HCl and 1.0 mM EDTA pH 8.0) overnight at 4 °C. Samples were incubated with a mixture of six enzymes (*Alu*I, *Cfo*I, *Hae*III, *Hinf*I, *Msp*I, and *Rsa*I) for 4 h at 37 °C and then separated on a 0.7% agarose gel overnight at 70 V. The gel was denatured in a solution of 1.5 M NaCl and 0.5 M Tris–HCl pH 8.0 for 20 min, rinsed in distilled water and dried for 2–3 h at 55 °C. The gel was then incubated in neutralizing solution (1.5 M NaCl, 0.5 M Tris–HCl pH 8.0) for 15 min and hybridized with a ^{32}P-labeled probe overnight at 42 °C. The gel was washed in 2× SSC for 15 min, then washed twice in 0.1× SCC plus 0.1% SDS for 10 min and rinsed again in 2× SCC. The gel was exposed to a PhosphorImager screen overnight and analyzed using a STORM 860 PhosphorImager (Molecular Dynamics).

Soft agar assay

Cells were treated for the indicated time points and plated in 12-well plates at 500 cells/well in 0.35% agar in culture medium over a 0.5% agar layer. Plates were incubated for about 10–14 days until colonies were large enough to visualize. Colonies were stained with 1 mg/ml MTT in culture media overnight. Colonies were counted, and the percent colony formation relative to the untreated cells was calculated. Experiments were done in triplicate.

Colony formation assay

Cells were plated at low density (250–500 cells per 10 cm^2 dish) on day 0. For treated cells, cells were given 2 treatments of 1 μM GRN163L or mismatch control (Geron Corporation) on days 0 and 4. Pictures were taken at day 7 using an inverted microscope (Ziess). Cells were then washed in PBS, fixed in 70% ethanol and stained with 20% Giemsa. The number of colonies was counted and expressed as percent growth of colonies: (number colonies/number of colonies of untreated cells) × 100.

Invasion assay

MDA-MB-231 or MDA-MB-435 cells were treated with 1 μM GRN163L or mismatch control every 3–4 days for 1 week. Untreated and treated cells were plated in Matrigel™-coated invasion chambers (BD Biosciences) and processed according to manufacturer's instructions. Briefly, cells were plated at $2.5–5.0 \times 10^4$ cells/well. HT1080 cells were used as a positive control, and BJ normal fibroblasts were used as negative control cells. Chemoattractant was added to the lower chambers: culture media plus 10% FBS was used for the MDA-MB-231 cells, and HT1080 culture media was used for the MDA-MB-435 cells. Cells were incubated for 22 h at 37 °C, 5% CO_2. Cells were removed from the top chamber using cotton swabs, and the cells that penetrated through the Matigel™ to the underside surface of the membrane were washed, fixed in 70% ethanol for 30 min, stained in 0.1% crystal violet for 5 min and rinsed. The membranes were mounted on slides. Random fields were chosen, and the number of cells that invaded through the Matrigel™ and migrated through the control inserts were counted. The percent invasion was calculated by: (mean number of cells invading through Matrigel™/mean number of cells migrating through control insert) × 100. The percent of control was calculated by: (percent invasion of the sample/percent invasion of untreated cells) × 100. Experiments were done in triplicate.

Oligonucleotide chemistries

N3′ → P5′ thio-phosphoramidate (NPS) oligonucleotides were prepared on 1 μmol scale using ABI 394 synthesizers as previously described [22], except that step-wise sulfurization during synthesis of NPS compounds was done with 0.1 M phenylacetyl disulfide (PADS) in CH_3CN/2,6-lutidine (1/1, v/v), 5 min, after the coupling step. Additionally, acetic anhydride was replaced by *iso*-butyric anhydride in the capping reagent. The 3′-aminonucleoside-containing 5′-phosphoramidites and CPG-based solid supports were purchased from Annovis Inc. (now Transgenomic Inc.). Oligonucleotide compounds were analyzed and purified, if needed, by ion exchange or reversed phase (RP) HPLC, and then desalted by either gel filtration on NAP-10 columns (Pharmacia), or by precipitation with cold ethanol (~5–7 volumes) from ~0.5 to 1.0 M NaCl solutions. The isolated compounds were characterized by ^{31}P NMR, mass spectrometry, polyacrylamide gel electrophoresis (PAGE), and by analytical RP HPLC, (C18 column, 1%/min CH_3CN gradient in 0.1 M triethylammonium acetate, pH 7.2). The RP HPLC retention times for GRN163 and GRN163L typically were 13.2 and 22.3 min, respectively, reflecting higher lipophylicity of the lipid-conjugated molecule – GRN163L.

Results

Effect of GRN163L on cellular proliferation

The MDA-MB-231 and MDA-MB-435 cells are both estrogen receptor- and progesterone receptor-negative cell lines that were derived from infiltrating ductal breast carcinoma [24]. Both of these cell lines express active telomerase. During treatment of MDA-MB-231 and MDA-MB-435 breast cancer cells, telomerase activity begins to decrease at about 48 h and remains inhibited for up to 6 days following the addition of a single 1 μM dose of GRN163L (data not shown). Therefore, MDA-MB-231 cells (Figure 1(a)) or MDA-MB-435 cells (Figure 1(b)) were treated with increasing doses of GRN163L for 72 h, and telomerase activity was analyzed using the TRAP (telomere repeat activity protocol) assay. TRAP activity was essentially completely inhibited at 1 μM GRN163L; therefore, we chose this dose for the long-term treatment studies.

To examine the effect of GRN163L on cellular growth, MDA-MB-231, MDA-MB-435 or normal BJ fibroblast cells were treated every 3–4 days, with no more than 4 days between treatments, with 1 μM GRN163L for 6 weeks (Figure 1(c)). Cells were collected and counted each week. The population doubling was calculated by: log (number of cells collected/number of cells initially plated)/log 2. While normal cells and the MDA-MB-231 cells continued growing in culture, the MDA-MB-435 cells showed decrease proliferation and senesced by week 6. Continued treatment of the MDA-MB-231 cells (upto 12 weeks) eventually resulted in decrease growth rate. The growth of normal BJ fibroblasts was not affected by long-term GRN163L treatment up to 6 weeks of continuous treatment. Mismatch controls were used in all experiments and had no effect on the cell growth of either cell line.

GRN163L treatment results in progressive telomere shortening

To determine if the inhibition of telomerase also resulted in progressive telomere shortening, evaluation of TRF length (telomere restriction fragment) was conducted. As shown in Figure 2, long-term continuous treatment of cells with GRN163L results in progressive telomere shortening. Treatment of cells with the mismatch control did not have an affect on telomere length shortening.

GRN163L treatment results in loss of colony formation in soft agar

To examine the tumorigenic potential of cells treated with GRN163L, cells were treated for varying times (2–10 weeks) and then plated in soft agar and incubated for 10–14 days. Cells were fed once per week with fresh

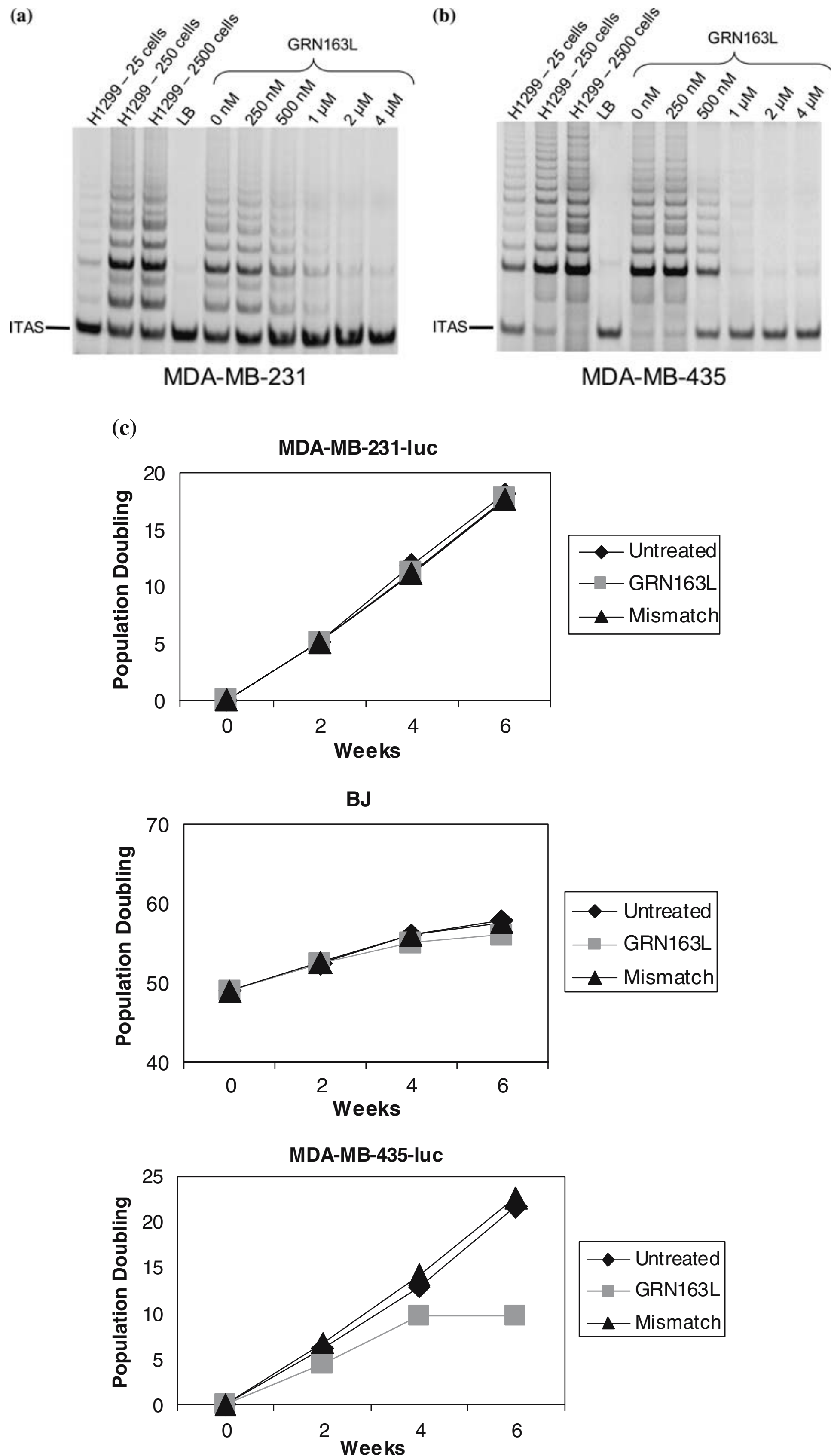

Figure 1. Growth rate of cells treated with GRN163L. (a and b) Cells were treated with a range of doses of GRN163L for 72 h. Cells were collected and telomerase activity was measured for 2500 cell equivalents per lane using TRAP assay (Cy5-labeled TS primer). Products were resolved on 10% polyacrylamide gels and processed using PhosphorImaging. H1299 cells were used as positive controls, and lysis buffer alone was used as a negative control. (c) Cells were treated with 1 μM GRN163L or mismatch control every 3–4 days for the indicated number of weeks. Each week cells were collected, counted and then replated and treated. The population doubling was calculated each week by: log (number of cells collected/number of cells initially plated)/log 2.

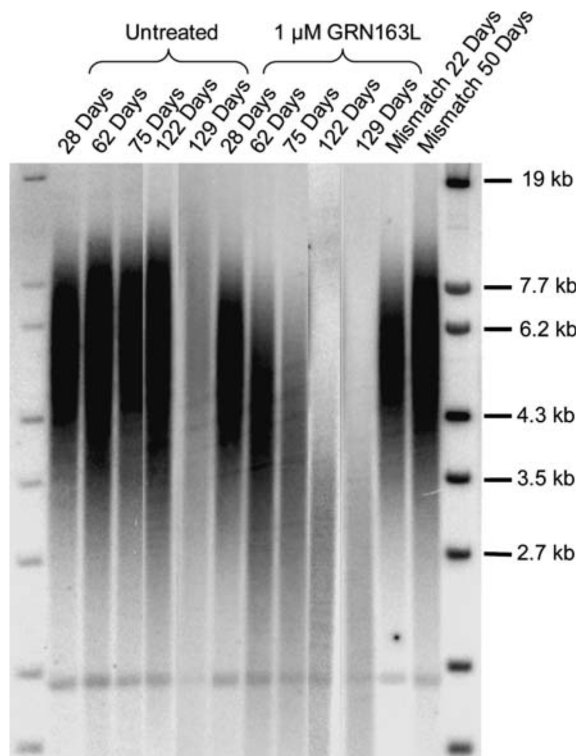

Figure 2. TRF analysis of MDA-MB-231 cells treated with GRN163L or mismatch control. Cells were treated with 1 μM GRN163L or mismatch control every 3–4 days for the indicated number of weeks. Each week samples were collected for TRF analysis at 1×10^6 cells/sample. TRF lengths are expressed as kilobase pairs.

medium (GRN163L was not added to the media). The GRN163L-treated MDA-MB-435 cells showed decreased ability to form colonies in soft agar following 2 weeks of GRN163L treatment prior to plating in soft agar, while the untreated cells or mismatch treated cells were still able to form colonies (Figure 3(a)). The MDA-MB-231 cells treated with GRN163L also began to show diminished ability to form colonies; however, this occurred after prolonged treatment (7 weeks or more) with GRN163L (Figure 3(b)). While both of these cell types show decreased colony formation, the cells were still capable of growth in cell culture long after they were unable to form colonies in soft agar. While an explanation for this apparent paradoxical result is yet unknown, one possibility is that the loss of growth in soft agar but not in cell culture is due to rapid telomere deletion events in a small percentage of cells, even when bulk telomere length is still long, leading to an overall DNA damage response independent of average telomere length of the entire population of cells. Another possibility is that telomerase may have an unknown telomere-independent function that may affect the tumorigenicity of cells following its inhibition. Moreover, cells that are treated for 10 weeks with GRN163L and then taken off the drug for 1–2 weeks regain the ability to form colonies in soft agar (Figure 3(c)), supporting the idea that it may be an acute DNA damage response affecting growth in soft agar.

Cells treated with GRN163L do not form colonies in colony formation assay

MDA-MB-435 cells were plated at low density (250–500 cells per 10 cm^2 dish) and incubated for 7–10 days under different conditions. For treated cells, GRN163L or mismatch control (1 μM each) was added to the media when cells were plated and every 3–4 days thereafter until the end of the experiment. Cells that were grown in the presence of GRN163L were completely unable to form colonies, whereas untreated or mismatch-treated control cells were not affected (Figure 4). Again, this effect occurs immediately, when the bulk telomere length is still quite long, suggesting that these results may be due to a DNA-damage response following the inhibition of telomerase, or a novel function of telomerase independent of telomere capping.

GRN163L treatment inhibits MDA-MB-231 and MDA-MB-435 invasion through matrigel

To examine the invasion ability of breast cancer cells treated with GRN163L, MDA-MB-231 and MDA-MB-435 cells were exposed to GRN163L or to mismatch control compound for 1 week prior to plating on Matrigel™-coated invasion chambers. Cells were allowed to invade for 22 h. Treatment with GRN163L inhibited invasion even only after 1 week of exposure to the compound, while either untreated, mismatch-treated, or positive control HT1080 cells were still able to invade. Similar to the findings from the soft agar and clonal efficiency assays, rapid inhibition of invasion occurred when the average telomere length was still relatively long and the cells were still growing well in monolayer cell culture. Therefore, these results suggest that GRN163L may be an important treatment option for invasion and metastasis, rather than or in addition to treating the tumor growth only (Figure 5).

Discussion

The results herein show that GRN163L is an effective and specific telomerase inhibitor, leading to telomere shortening in breast cancer cells, greatly decreased growth in clonal plating assays, and decreased colony formation in soft agar, as well as inhibition of cellular invasion in Matrigel™. Telomerase is a highly attractive target for chemotherapeutic approaches since it is expressed in almost all cancer cells but not in normal somatic cells. One of the major challenges for potential application of telomerase inhibitors as chemotherapeutic agents is believed to be the time requirement that might be needed before cell growth is affected. It was suggested and demonstrated with some telomerase inhibitors that there is a time lag from the initial onset of telomerase inhibition to the moment when massive cellular senescence and death occurs due to telomere erosion. For example, we showed that several weeks are

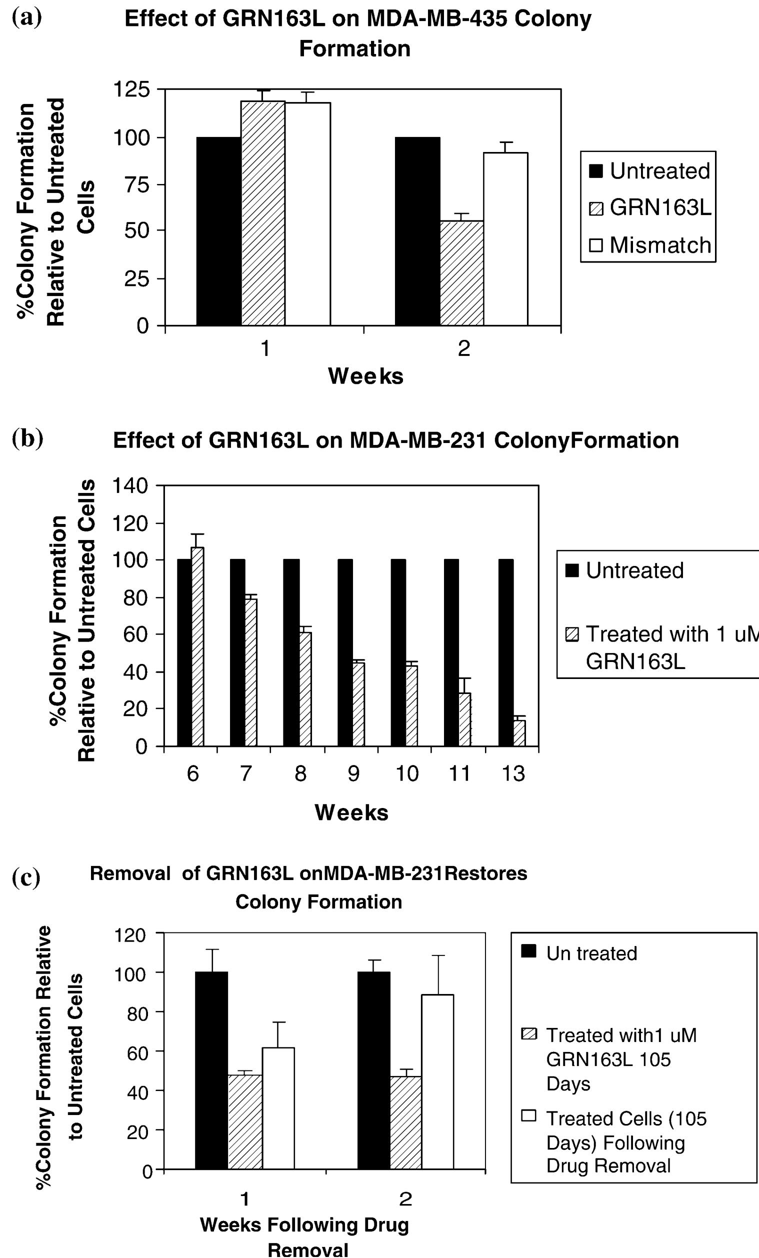

Figure 3. Colony formation in soft agar of cells treated with GRN163L. MDA-MB-435 cells (a) or MDA-MB-231 cells (b) were treated with 1 μM GRN163L or mismatch control for the indicated number of weeks. Each week cells were collected and plated in soft agar (fed once per week without the addition of drug). After 10–14 days, colonies were stained with MTT, and the number of colonies was counted. The percent colony formation relative to untreated cells was calculated. (c) MDA-MB-231 cells were treated in culture with 1 μM GRN163L for 105 days; these cells were then continued in culture for 2 weeks in the absence of GRN163L. Each week cells were collected and plated in soft agar. Again, following 10–14 days of incubation, colonies were stained with MTT and counted. The percent colony formation relative to untreated cells was calculated.

required before significant telomere shortening and decreased proliferation were observed in cell culture (Figures 1 and 2). An explanation for the difference in the growth response between the MDA-MB-231 and MDA-MB-435 cells following treatment with GRN163L for 6 weeks is not known, as both cell lines express active telomerase and have similar telomere lengths (data not shown for MDA-MB-435). It is possible that there are some telomeres on a few chromosomes are shorter in the MDA-MB-435 cells that lead to an earlier growth arrest even though the average telomere lengths in both cells lines are similar. Nonetheless, we show that following only 1 week exposure to 1 μM of GRN163L, the ability of both of these cells to invade through Matrigel™ is almost completely inhibited. This occurs when the average telomere length is still long and, as a population, telomeres are not yet critically shortened as a result of telomerase inhibition. This finding was unexpected and may indicate novel functions of telomerase or telomerase RNA that are independent of average telomere length.

The rapid effects of inhibited cell growth in soft agar, inability to form colonies when plated at low density, and inhibited migration may also be due to catastrophic

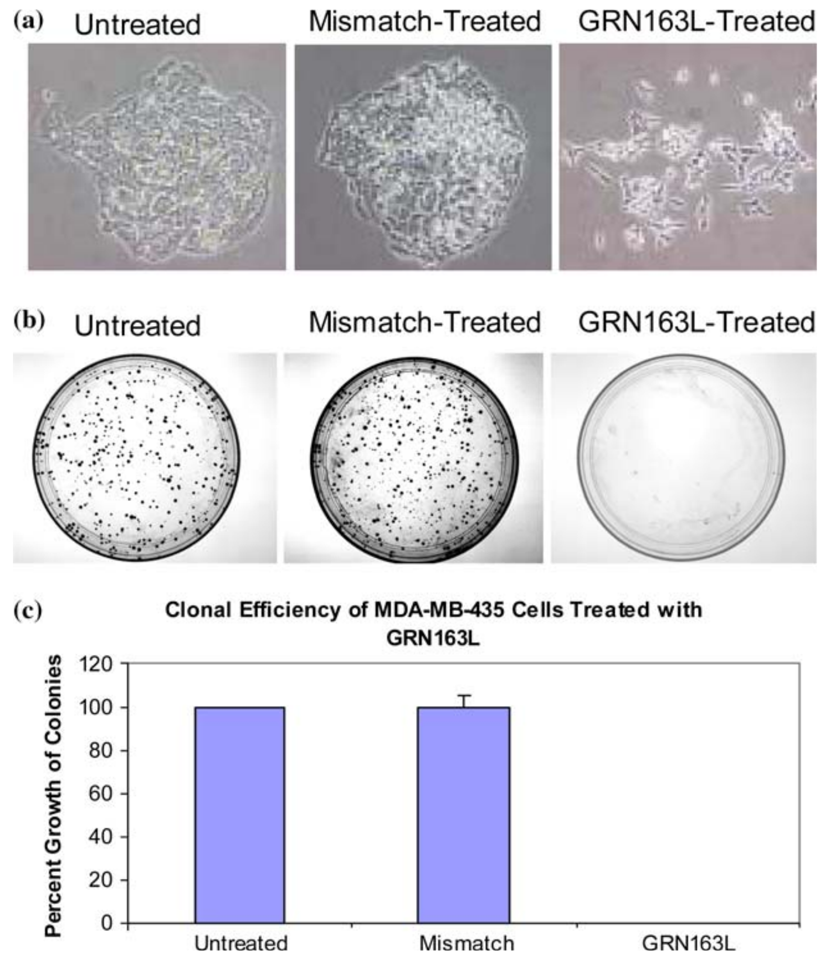

Figure 4. Effect of GRN163L on colony formation in clonal efficiency assay. Cells were plated at low density (250–500 cells per 10 cm^2 dish), in the presence of 1 μM GRN163L or mismatch control (given every 3–4 days), and colonies were allowed to form for 7–10 days. Pictures were taken at day 7 (a), and colonies were then stained with Giemsa (b) and counted. (c) The number of colonies was expressed as percent growth of colonies: (number of colonies/number of colonies of untreated cells) × 100.

or rapid telomere deletion events that take place in the presence of telomerase inhibition. In the presence of GRN163L-induced telomerase inhibition, this uncapping of telomeres may result in a DNA damage-like response affecting the ability of tumor cells to invade through Matrigel™. Nevertheless, these results indicate either a novel functional role for telomerase that is independent of telomere capping or a response due to DNA damage. Both hypotheses warrant further investigation of the observed GRN163L effects.

The finding that GRN163L inhibits the ability of breast cancer cells to invade suggests that alternative treatment options for breast cancer should be explored, such as using telomerase inhibitors following surgical removal of tumors. This option may greatly decrease the time required to see therapeutic results as seen with telomerase inhibitors alone, since the bulk tumor mass will be surgically removed, and the time needed for telomerase inhibitors to decrease the size of the tumor will no longer be a major challenge. Therefore, patients with minimal residual disease may be treated with telomerase inhibitors, such as GRN163L, to prevent any further tumor growth and importantly, formation and spread of metastasis. Moreover, since the invasion of cells was an immediately observed outcome of administration of GRN163L, and it appears to be independent of gross telomere shortening, these results suggest a possibility in treating the metastasis of any tumor type regardless of telomere lengths that vary with each different tumor type. Therefore, GRN163L may be especially useful in combination with other standard chemotherapeutic drug options.

In summary, we have shown that the telomerase inhibitor, GRN163L, is an anti-tumorigenic agent, inhibiting the growth of breast cancer cells in soft agar and decreasing their invasive potential. These data will aid in the planning of *in vivo* studies and may ultimately provide knowledge for the design of possible human Phase I clinical trials to evaluate GRN163L as a therapeutic agent against human breast cancer.

Acknowledgements

The authors thank William Walker for technical assistance with TRF analysis. The work at UTSW is supported by Geron Corporation, Menlo Park, CA and CA70907. We also thank Dr. Krisztina Pongracz and

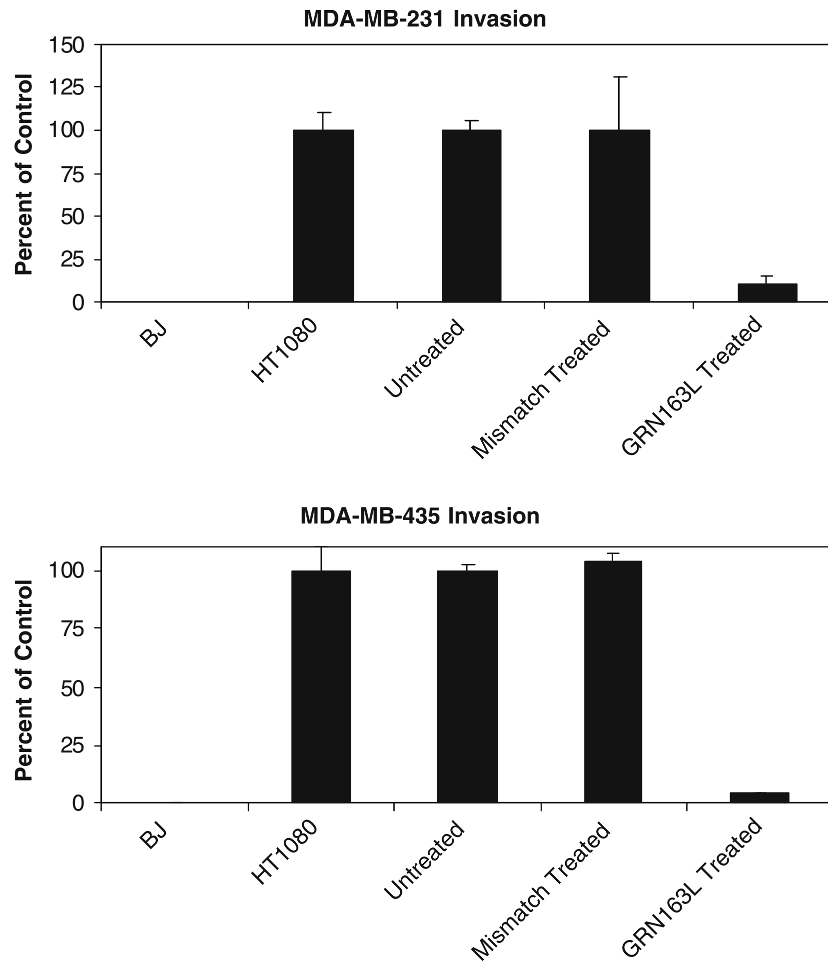

Figure 5. Invasion of breast cancer cells treated with GRN163L. MDA-MB-231 cells (a) or MDA-MB-435 cells (b) were treated with 1 μM GRN163L or mismatch control for 1 week. Cells were then plated in Matrigel™-coated invasion chambers for 22 h. Culture media with 10% FBS was used for MDA-MB-231 cells, and HT1080 culture media was used for the MDA-MB-435 cells. BJ cells were used as a negative control, and HT1080 cells were used as a positive control. Cells that invaded through were stained with 0.1% crystal violet, and cells were counted in random fields that were chosen. The percent of control was calculated by: (percent invasion of the sample/percent invasion of untreated cells) × 100.

Daria Zielinska for help with the initial oligonucleotide preparation.

References

1. Blackburn EH: Switching, signaling at the telomere. Cell 106: 661–673, 2001
2. Harley CB: Telomere loss: mitotic clock or genetic time bomb? Mutat Res 256: 271–282, 1991
3. Harley CB, Futcher AB, Greider CW: Telomeres shorten during ageing of human fibroblasts. Nature 345: 458–460, 1990
4. Greider CW, Blackburn EH: Telomeres, telomerase and cancer. Sci Am 274: 92–97, 1996
5. Wright WE, Piatyszek MA, Rainey WE, Byrd W, Shay JW: Telomerase activity in human germline and embryonic tissues and cells. Dev Genet 18: 173–179, 1996
6. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE: Extension of life-span by introduction of telomerase into normal human cells. Science 279: 396–352, 1998
7. Kiyono T, Foster SA, Koop JI, McDougall JK, Galloway DA, Klingelhutz AJ: Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. Nature 396: 84–88, 1998
8. Yang J, Chang E, Cherry AM, Bangs CD, Oei Y, Bodnar A, Bronstein A, Chiu CP, Herron GS: Human endothelial cell life extension by telomerase expression. J Biol Chem 274: 26141–26148, 1999
9. Shay JW, Bacchetti S: A survey of telomerase activity in human cancer. Eur J Cancer 33: 787–791, 1997
10. Mokbel K: The role of telomerase in breast cancer. Eur J Surg Oncol 26: 509–514, 2000
11. Shay JW, Wright WE: Telomeres and telomerase: implications for cancer and aging. Radiat Res 155: 188–193, 2001
12. Shay JW, Zou Y, Hiyama E, Wright WE: Telomerase and cancer. Hum Mol Genet 10: 677–685, 2001
13. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW: Specific association of human telomerase activity with immortal cells and cancer. Science 266: 2011–2015, 1994
14. Hiyama E, Gollahon L, Kataoka T, Kuroi K, Yokoyama T, Gazdar AF, Hiyama K, Piatyszek MA, Shay JW: Telomerase activity in human breast tumors. J Natl Cancer Inst 88: 116–122, 1996
15. Allred DC, O'Connell P, Fuqua SA: Biomarkers in early breast neoplasia. J Cell Biochem Suppl 17G: 125–131, 1993
16. Poremba C, Shroyer KR, Frost M, Diallo R, Fogt F, Schafer KL, Burger H, Shroyer AL, Dockhorn-Dworniczak B, Boecker W: Telomerase is a highly sensitive and specific molecular marker in fine-needle aspirates of breast lesions. J Clin Oncol 17: 2020–2026, 1999

17. Bieche I, Nogues C, Paradis V, Olivi M, Bedossa P, Lidereau R, Vidaud M: Quantitation of hTERT gene expression in sporadic breast tumors with a real-time reverse transcription-polymerase chain reaction assay. Clin Cancer Res 6: 452–459, 2000
18. Hoos A, Hepp HH, Kaul S, Ahlert T, Bastert G, Wallwiener D: Telomerase activity correlates with tumor aggressiveness and reflects therapy effect in breast cancer. Int J Cancer 79: 8–12, 1998
19. White LK, Wright WE, Shay JW: Telomerase inhibitors. Trends Biotechnol 19: 114–120, 2001
20. Herbert B, Pitts AE, Baker SI, Hamilton SE, Wright WE, Shay JW, Corey DR: Inhibition of human telomerase in immortal human cells leads to progressive telomere shortening and cell death. Proc Natl Acad Sci USA 96: 14276–14281, 1999
21. Herbert BS, Pongracz K, Shay JW, Gryaznov SM, Shea-Herbert B: Oligonucleotide N3′–>P5′ phosphoramidates as efficient telomerase inhibitors. Oncogene 21: 638–642, 2002
22. Asai A, Oshima Y, Yamamoto Y, Uochi TA, Kusaka H, Akinaga S, Yamashita Y, Pongracz K, Pruzan R, Wunder E, Piatyszek M, Li S, Chin AC, Harley CB, Gryaznov S: A novel telomerase template antagonist (GRN163) as a potential anticancer agent. Cancer Res 63: 3931–3939, 2003
23. Gryaznov S, Pongracz K, Matray T, Schultz R, Pruzan R, Aimi J, Chin A, Harley C, Shea-Herbert B, Shay J, Oshima Y, Asai A, Yamashita Y: Telomerase inhibitors-oligonucleotide phosphoramidates as potential therapeutic agents. Nucleos Nucleot Nucleic Acids 20: 401–410, 2001
24. Zhang X, Mar V, Zhou W, Harrington L, Robinson MO: Telomere shortening and apoptosis in telomerase-inhibited human tumor cells. Genes Dev 13: 2388–2399, 1999

Address for offprints and correspondence: Jerry W. Shay Department of Cell Biology, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, 75390-9039, Dallas, Texas, USA; *Tel.*: +214-648-3282; *Fax*: +214-648-8694; *E-mail*: Jerry.Shay@UTSouthwestern.edu