INTRODUCTION:
Human mesenchymal stem cells (hMSCs) are attractive candidates for tissue engineering. Recent studies have shown that over-expression of telomerase enhances the proliferative lifespan of hMSCs while maintaining multilineage differentiation capacity. Telomerase is a ribonucleoprotein composed of two essential components, an integral RNA template (TR) and a catalytically active reverse transcriptase subunit (hTERT), that function together to elongate telomeres (1). TR is present in all cells; hTERT is expressed only in immortal and malignant cells. Although over-expression of hTERT can produce “immortal” cells with active telomerase, the mechanism(s) by which telomerase activity affects the cells’ lifespan is unclear. In tumor cells, telomerase activity is thought to convey immortality through maintenance of telomere length. However, in hair follicle stem cells, hTERT expression has been shown to promote proliferation of resting stem cells independent of telomere elongation (2). This apparent dichotomy motivated us to investigate whether immortalization of hMSCs by hTERT is dependent only upon maintenance of telomere length or upon another action of the hTERT protein. To elucidate the primary mechanism of hMSC immortalization, we used a selective inhibitor of telomere elongation. Developed as an anti-cancer drug, GRN163L is a thio-phosphoramidate oligonucleotide that targets the template region of TR, and competitively inhibits the interaction between hTERT and hTR. Thus, GRN163L blocks telomere elongation without interfering with other hTERT activity.

METHODS:
MSC isolation and culture: hMSCs were harvested from femoral bone marrow reamings after Percoll gradient separation. Cells were maintained in high glucose DMEM with 10% FBS, and passaged 1:4 at 80-90% confluence.

Inducible hTERT retrovirus construction: A RevTRE-hTERT construct was created by ligating the hTERT gene (kindly provided by Dr. Robert Weinberg, Whitehead Institute) into the multiple cloning site of the RevTRE vector (Clontech, CA). RevTRE-hTERT and RevTet-on plasmids were transfected into the packaging cell line PT67 to generate the recombinant retroviruses.

Generation of hTERT-expressing hMSCs (hTERT-hMSCs): hMSCs were sequentially infected with RevTet-on retrovirus and hTERT-hMSCs over-expression of hTERT was confirmed by Western blot analysis. Cells were harvested from femoral bone marrow reamings after Percoll gradient separation. Cells were maintained in high glucose DMEM with 10% FBS, and passaged 1:4 at 80-90% confluence.

RESULTS:
GRN163L was known to target hTR and inhibit telomere elongation and underwent senescence much earlier than untreated cells. hTERT-hMSCs were divided into two groups, one treated with 0.1 uM GRN163L, and one not treated. After one week of GRN163L treatment, telomerase activity was significantly inhibited in GRN163L treated hTERT-hMSCs (Fig.1). GRN163L treated cells continued to proliferate for 3 population doublings (PDs) after treatment initiation, at which point their proliferation slowed, and they reached replicative senescence at 9 PDs. Untreated hMSCs continued to proliferate at a steady rate for at least 40 PDs (Figs.2&3). Consistent with previously reported data (3), treatment with GRN163L at the concentrations used in these experiments did not have any measurable toxic effects on hMSCs (data not shown).

DISCUSSION:
We have shown that GRN163L blocks telomerase activity in hTERT-hMSCs. Cells treated with GRN163L stopped proliferating and underwent senescence much earlier than untreated cells. GRN163L is known to target hTR and inhibit telomere elongation without altering the hTERT component of telomerase. Therefore, in contrast to what has been reported for hair follicle stem cells, hTERT immortalization of hMSCs appears to be related to telomere elongation activity. However, these experiments do not exclude the possibility that other activities dependent upon TR-hTERT association are also critical. Additional studies are needed to further characterize effects of telomerase activity in hMSCs.

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GRN163L INHIBITS PROLIFERATION AND TELOMERASE ACTIVITY IN IMMORTALIZED HUMAN MESENCHYMAL STEM CELLS

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