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Inhibiting Telomerase using Imetelstat

- A Novel Oligonucleotide to Treat Cancer

Department of Applied Sciences

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Declaration

I certify that this literature review does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any college; and to the best of my knowledge and belief it does not contain any material previously published or written by another person where due reference is not made in the text.

Acknowledgement

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Date

Abstract

Telomeres, the conserved guanine-rich sequences at the end of eukaryotic linear chromosomes, shorten with every cell division until a critical short length, known as the Hayflick limit, is reached. When this occurs, the cell either undergoes cell growth arrest (replicative senescence) or programmed cell death (apoptosis). Therefore, the maintenance of telomere length is related to cellular immortalisation, a hallmark of cancer. In fact, in over 85% of cancers the reverse transcriptase enzyme telomerase is highly expressed. This enzyme is capable of extending telomere length, thereby contributing to telomere length maintenance. It consists of two main subunits: TERT (hTERT in humans) and TERC (hTERC in humans). HTERT acts as the catalytic subunit while hTERC contains the RNA template required for the synthesis of telomeric sequences. Since telomerase is expressed in most cancerous cells but absent or only present in low levels in most somatic cells, it presents an ideal target for anti-cancer therapy. This literature review examines current approaches to the inhibition of telomerase with a particular focus on the novel oligonucleotide GRN 163L, also known as Imetelstat. This compound was created by Geron and is the first telomerase inhibitor in clinical development. Imetelstat has passed several preclinical trials *in vitro* and *in vivo*, causing telomerase inhibition and telomere shortening which lead to cells undergoing replicative senescence or apoptosis. As a result, GRN 163L is currently investigated in several phase I and II clinical trials. This review will outline some of the up-to-date findings.

Key words: Cancer, cell cycle, telomeres, genomic stability, end-replication problem, replicative senescence, apoptosis, telomerase, hTERC, hTERT, oligonucleotide, GRN 163L, Imetelstat

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1. Introduction

“If recent trends in major cancers are seen globally in the future, the burden of cancer will increase to 22 million new cases each year by 2030.”

(Cancer Research UK, 2012).

Cancer is an umbrella term for a large group of diseases, all of which are characterised by the uncontrolled proliferation of abnormal cells in a localised area of the body (National Cancer Institute, 2005). Cancer can develop in various body tissues and while each cancer type has its own distinctive characteristics, the processes that give rise to cancer are similar in all cancer types. Cancer development is initiated when a mutation is introduced into one cell that is nonetheless allowed to proliferate. This cell's progeny develop and accumulate further mutations, giving rise to abnormal cells that exhibit inappropriate proliferation and develop into a tumour. This tumour may remain in the tissue where it originated or may invade neighbouring body parts and spread to other tissues and organs in a process known as metastasis. A tumour can become life-threatening when its size and growth interferes with normal tissue and organ function.

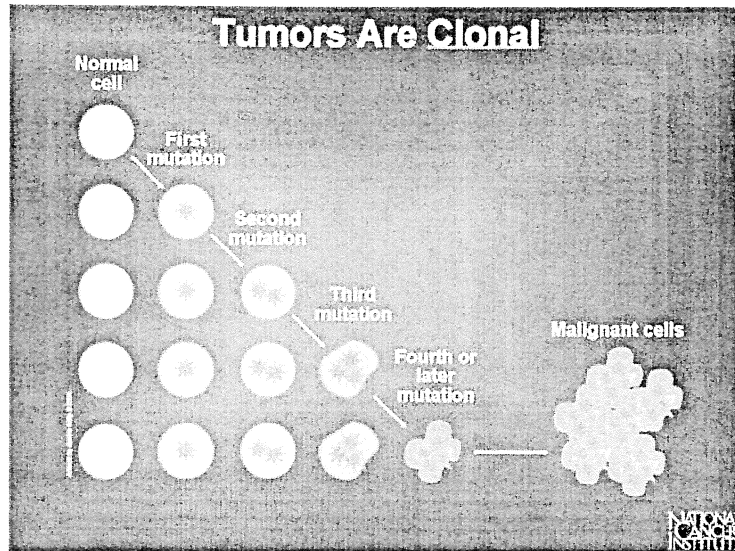


Figure 1: Tumour Development (National Cancer Institute, 2005). Diagram showing how a single, ancestral cell can give rise to tumour development through mutating, continued proliferation and continued accumulation of further mutations (National Institutes of Health & National Cancer Institute, 1999).

According to Cancer Research UK (2012), an estimated 12.7 million new cancer incidences were observed in 2008 and approximately 7.6 million people died as a result of the disease in that year. If current rates do not decrease, cancer incidences are estimated to increase to 22 million new cases every year by 2030.

More recent figures for invasive cancer incidences and mortalities in Europe can be viewed in figure 2 (National Cancer Registry Ireland, 2013a).

Estimated cancer incidence and mortality in Europe 2012, all invasive cancers (excluding non-melanoma skin)

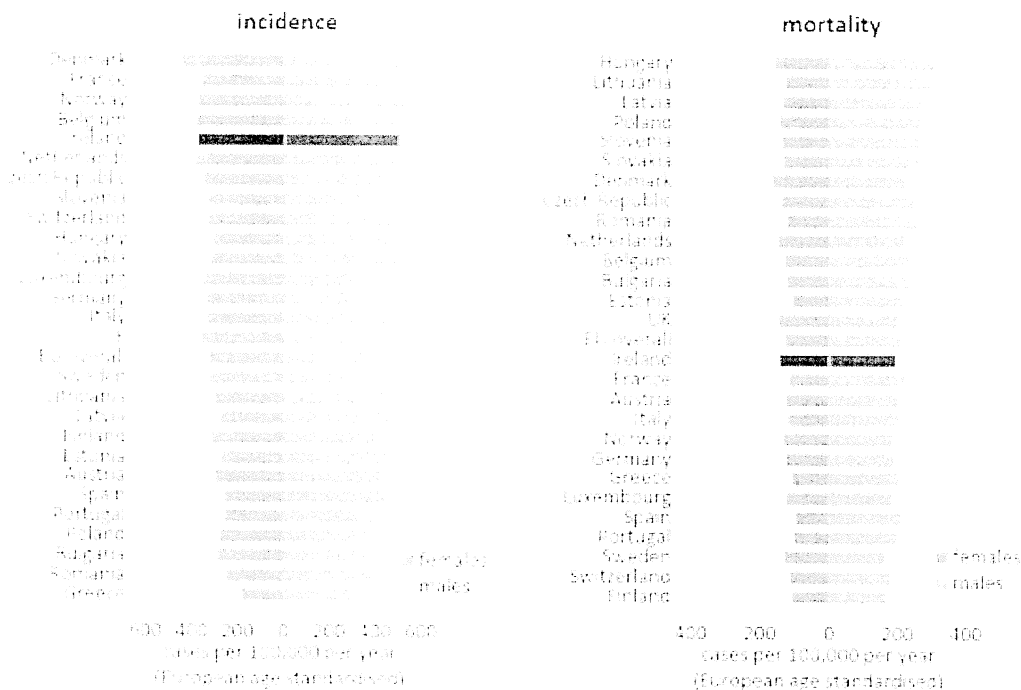


Figure 2: Invasive cancer incidence and mortality rates in Europe in 2013 (National Cancer Registry Ireland, 2013a). Ireland’s invasive cancer incidence rates were found to be among the highest in Europe. The mortality rates established for Ireland were similar to those determined for the UK. The Irish ratio of mortality/incidence was comparable to the EU rate with circa 4 deaths for every 10 incident cases.

In Ireland, cancer was established to be the second most common cause of death in 2010 with 8,316 people dying of the disease that year (National Cancer Registry, 2013a). This corresponded to circa 30% of all deaths in Ireland. Figure 3 shows national cancer rates by county in Ireland from 1994-2010 (National Cancer Registry, 2013b).

ALL INVASIVE CANCERS (C00-C43, C45-C96) 1994-2010

*excludes non-melanoma of skin

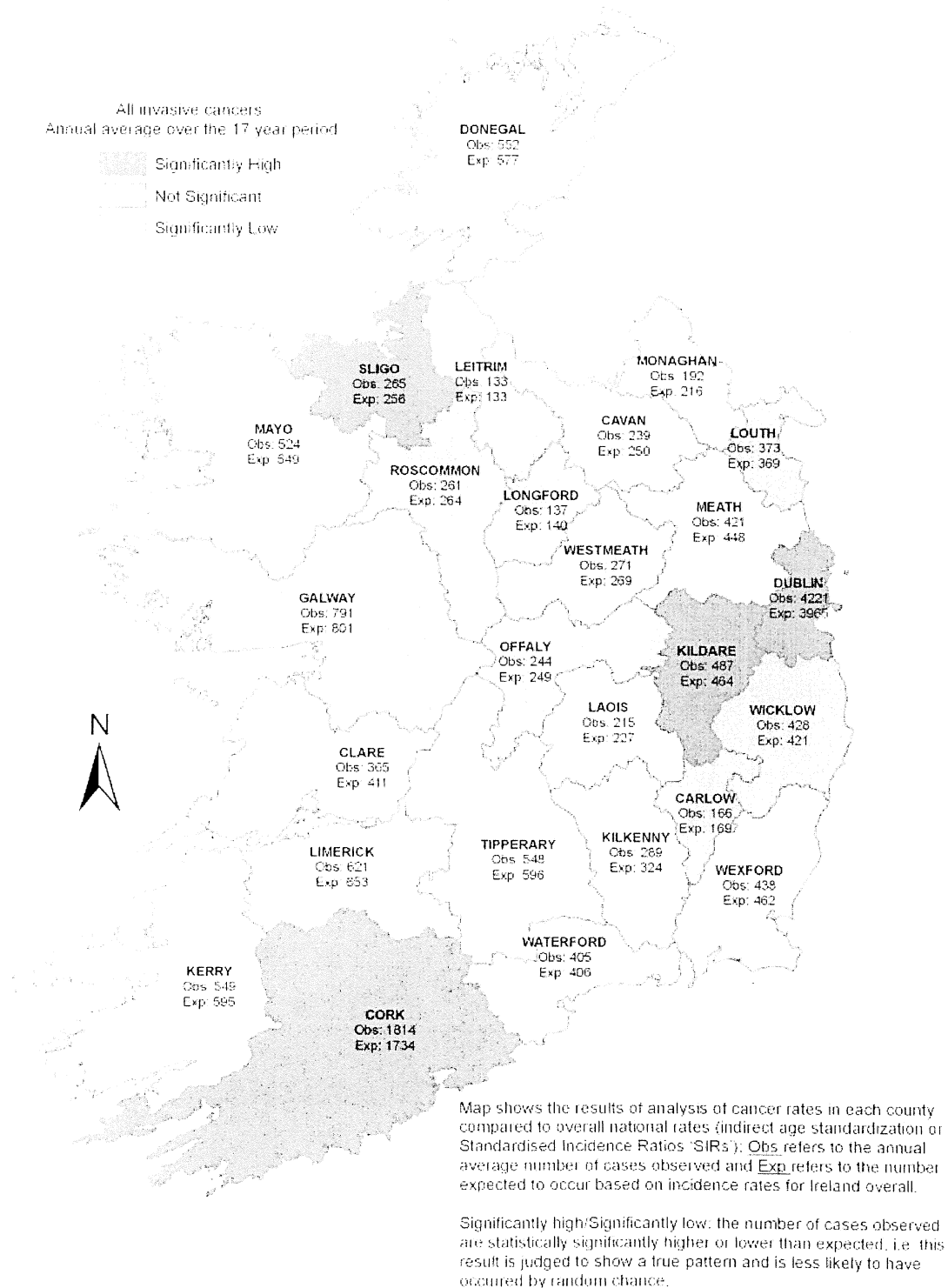


Figure 3: Cancer in Ireland (National Cancer Registry, 2013b). Diagram showing cancer incidence rates in Ireland by county from 1994-2010.

Cancer research therefore is aimed at treating this disease successfully and improving patients' quality of life. It primarily focuses on identifying particular biomarkers or targets for therapeutic developments in order to identify and target cancer cells specifically and minimise damage to normal tissues as caused by some conventional anti-cancer therapies such as chemotherapy (Jeronimo & Henrique, 2014). Recently, cancer research has investigated telomerase as a possible therapeutic target since this enzyme is thought to be responsible for the ability of cancer cells to proliferate indefinitely (Cunningham *et al.*, 2006). Inhibition of telomerase is therefore believed to halt cancer cell growth and possibly lead to programmed cell death, known as apoptosis, in cancer cells.

In the following overview, the growth and proliferation of normal cells will be discussed as well as how these processes differ in cancer cells. Furthermore, the role of telomeres, the highly conserved regions of DNA at the end of eukaryotic linear chromosomes, in normal and cancerous cells will be examined as well as the importance of the telomerase enzyme in the maintenance of telomere length, particularly in cancer cells. Additionally, this review will look at different approaches to telomerase inhibition with a particular focus on the novel therapeutic treatment Imetelstat. Finally, a short conclusion will be given.

1.1 The Cell Cycle in Normal and Cancerous Cells

In order to understand how tumour growth occurs, the following section will describe the cell cycle and its regulation in normal, non-cancerous eukaryotic cells compared to abnormal, cancerous cells.

One of the principles of the cell theory is the idea that new cells can only arise from other existing cells by a process known as cell division, which differs in prokaryotes and eukaryotes (Karp, 2008). For the purposes of this review, only the activities involved in the eukaryotic cell cycle will be discussed.

Cell division is the process that allows the development of a multicellular organism, such as a human, from a single-celled zygote. While some highly specialised cells cease to divide after they have differentiated (e.g. neurons), others proliferate at a relatively high rate and thus renew certain tissues of the body (e.g. cells of intestinal wall lining). The cell cycle is a precisely timed and controlled sequential process leading to cell division and the formation of two identical daughter cells, each of which contain genetic material identical to the parent cell (Lodish *et al.*, 2013).

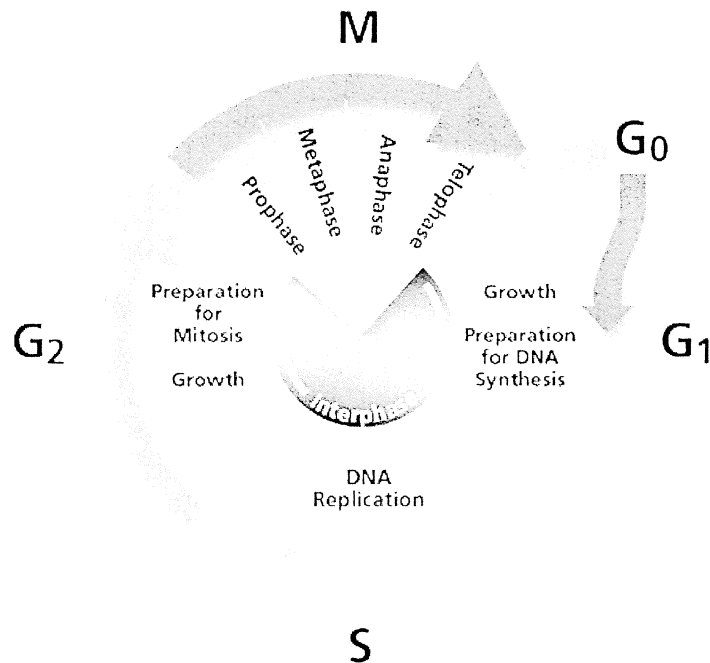


Figure 4: Stages of the eukaryotic cell cycle (BD Biosciences, 2013). In G_1 , mRNAs and proteins required for DNA synthesis are produced, cell organelles are duplicated and the cell increases in size (Karp, 2008; Lodish *et al.*, 2013). When this process is complete, the cell reaches a point in G_1 known as START, the crossing of which commits the cell to cell division. During the S phase, the cell undergoes DNA replication and chromosome duplication. In G_2 , the cell grows and prepares for mitosis, during which the actual cell division takes place. Cycling cells enter the G_1 phase again after completing the M phase.

Generally, the eukaryotic cell cycle can be divided into two main stages: Mitosis or M phase and Interphase (Karp, 2008). The former can be further subdivided into mitosis and cytokinesis. Mitosis involves several steps during which the cell's chromosomes are duplicated and subsequently separated, while cytokinesis is the physical process that divides the entire cell into two daughter cells. Interphase includes all the activities required for the cell to prepare for mitosis and can be divided into three stages: G_1 (first gap) phase, S (synthesis) phase and G_2 (second gap) phase. While cycling cells can re-enter G_1 after completing mitosis, most cells cease to proliferate once they have differentiated. These cells are capable of living for days, weeks or in some cases even the entire lifetime of the organism without

proliferating again (e.g. neurons). Such cells are referred to as being postmitotic; they exit the cell cycle in the G_1 phase to enter a phase known as G_0 . Some cells may re-enter the cell cycle again at a later stage, a process highly regulated in order to control cell proliferation.

In order to produce two genetically identical daughter cells, it is important that the cell cycle stages take place in the correct order, that each phase is fully completed before the next one commences and that cells carrying damaged DNA are not allowed to continue cycling until the damage is fixed or the cell undergoes programmed cell death (apoptosis) if the damage is extreme. The cell's progression through the cell cycle is regulated by protein complexes known as cyclin-dependent kinases (CDKs) as well as surveillance mechanisms known as checkpoint pathways. Cancer cells often show mutations in these complex mechanisms that regulate and control the cell cycle in eukaryotes. For example, in 80% of human cancers, the migration through the division-committing START stage during G_1 is misregulated by affecting complexes such as CDKs and Rb proteins which control this mechanism. P53 is another important protein that is thought to be affected in most if not all human tumours by either harbouring a mutation in the p53 gene itself or mutations being present in proteins that control the activity of p53. This protein is important in tumour suppression as it stimulates cell cycle arrest in G_1 and G_2 when DNA damage is detected and it initiates DNA damage repair or apoptosis if the damage is too severe. Mutations in the gene coding for p53, leading to a loss of the protein's proper functioning is prevalent in more than 50% of human cancers; in some cancers p53 itself is not affected but other proteins such as Mdm2 that inhibit the activity of p53 are active. When these cell cycle checkpoints are disrupted, the cell continues to traverse the cell cycle irrespective of possible DNA damage being present, thereby

permitting tumour growth. In addition to the cell cycle regulators and checkpoints, further important features of the cell that seem to play a pivotal role in the cell's ability to proliferate are telomeres and their elongation by telomerase. Both of these will be discussed in the following sections.

1.2 Telomeres

In 1961, Leonard Hayflick and Paul Moorhead discovered that cells were only able to divide a limited number of times, a phenomenon now known as the Hayflick limit (Cunningham *et al.*, 2006). This stage of growth arrest after a period of regular cell proliferation is known as cellular senescence and the cell may subsequently remain metabolically active for a period of time. Today it is known that the limit of cellular replication is due to telomeres.

"Telomeres" is the name given to the highly conserved regions at the end of eukaryotic linear chromosomes that are made up of telomeric DNA and associated proteins (Satyanarayana *et al.*, 2004). Human telomeres are rich in the purine base guanine (G) and consist of the hexameric repetitive sequence unit (TTAGGG)_n. The subscripted n indicates that the quantity of these repeats varies among mammalian species, but in humans it generally ranges from 10-15 kilobases (kb). The majority of a telomere is double stranded; however, the very end is comprised of a 150-200 nucleotide-long 3' single stranded overhang that is also rich in G (Mocellin *et al.*, 2013; Satyanarayana *et al.*, 2004). This overhang bends and displaces a portion of the double stranded telomere structure to form higher order structures such as T- and D-loops.



Figure 5: Proposed telomeric T-loop and D-loop formation (adapted from DeLange, 2004). The 3' telomeric overhang is approximately 100-200 nucleotides long (DeLange, 2004). When a telomeric T-loop is formed, the 3' overhang bends back and displaces part of the double-stranded telomeric portion forming a displacement D-loop. Single-stranded telomeric DNA is bound by protein POT1 for telomere protection (Cunningham *et al.*, 2006). Other proteins involved in telomere binding are telomeric repeat binding factors (TRF) such as TRF1 and TRF2.

These structures are stabilised by the binding of telomere sequence specific protein complexes known as shelterin (DeLange, 2005). Each complex consists of six protein subunits, specifically TRF1, TRF2, TIN2, Rap1, TPP1 and POT1. The shelterin complex acts as a telomeric cap and its purpose is the protection of chromosomal ends from degradation, fusion and recombination as well as the stimulation of T-loop formation, maintaining telomere length and recruiting telomerase (Mocellin *et al.*, 2013; Buseman *et al.*, 2012).

Chromosomes that do not carry sufficiently long telomeres experience end-to-end fusions with other chromosomes and form multicentric chromosomes (Gisselsson *et al.*, 2001, cited in Satyanarayana *et al.*, 2004). This leads to genomic instability and the activation of DNA damage response mechanisms, such as cell cycle checkpoints, which generally results in p53-dependent induced senescence or apoptosis (Buseman *et al.*, 2012). Hence, to ensure survival and growth of a cell, the

maintenance of functional telomere length is crucial. However, with every cell division, the telomeric ends of chromosomes shorten until the Hayflick-limit is reached. This process is known as the end-replication problem and is discussed briefly in the following section.

1.3 The End-Replication Problem

The end-replication problem, first independently described by the scientists Alexey Olovnikov and James Watson, illustrates how with every DNA replication some bases at the very ends of chromosomes get lost, thereby shortening the telomeres until the Hayflick limit is reached (DeLange and Jacks, 1999).

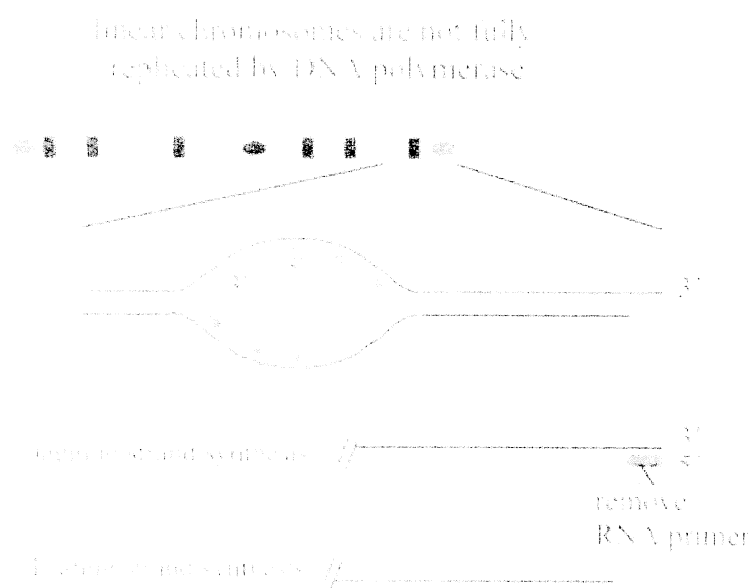


Figure 6: The End-Replication Problem (Chan & Blackburn, 2004). While the leading strand can be replicated without any problems, DNA polymerase is not able to replicate the very terminal end of the lagging strand, which leads to progressive telomere shortening with every cell division.

During DNA replication in the S phase of the cell cycle, DNA polymerase extends RNA primers to synthesise an identical copy of the double stranded DNA (Chan & Blackburn, 2004). Since DNA synthesis in the leading strand ($5' \rightarrow 3'$) follows the direction of the replication fork, this strand can be replicated continuously to the very end. However, the lagging strand (overall $3' \rightarrow 5'$) is synthesised in the opposite direction of the replication fork by the extension of RNA primers creating Okazaki fragments. Since the primers are subsequently removed, the resulting spaces are filled by DNA polymerase by extending the newly synthesised DNA and subsequent ligation. However, this process cannot be carried out for the gap left by the removal of the most terminal primer as no newly synthesised DNA precedes it. As a result, the telomere is shortened by 50-200 bases with every cell division leading to continuously shorter chromosomes (Cunningham *et al.*, 2006).

The shortening of telomeres can be counteracted by a reverse transcriptase enzyme known as telomerase which is capable of extending telomeric sequences.

1.4 Telomerase

Telomerase belongs to a family of enzymes known as reverse transcriptases (Chan & Blackburn, 2004). The telomerase holoenzyme consists of two main components: the reverse transcriptase protein subunit TERT (hTERT in humans) and the RNA subunit TERC (hTERC in humans) (Satyanarayana *et al.*, 2004). The telomerase RNA component acts as a template for the synthesis of telomeric sequences and binds to hTERT as well as other proteins associated with telomerase to form secondary structures such as pseudo-knots and CR7 domains. These structures are conserved and have been shown to carry specific functions regarding

correct telomerase functioning. hTERT on the other hand is the catalytic element of the enzyme. Analyses involving the alignment of reverse transcriptase sequences showed that hTERT is arranged further into domains that are conserved among reverse transcriptases as well as domains that are specific to telomerase. In more recent studies it was also identified that the N terminus of hTERT is involved in enzymatic activity and binding to hTERC, whereas the C terminus plays a role in recruiting the enzyme to the telomere. While hTERC is expressed in all human cells, telomerase activity depends on the presence of hTERT, which is only expressed in cells that require telomerase activity (Buseman *et al.*, 2012).

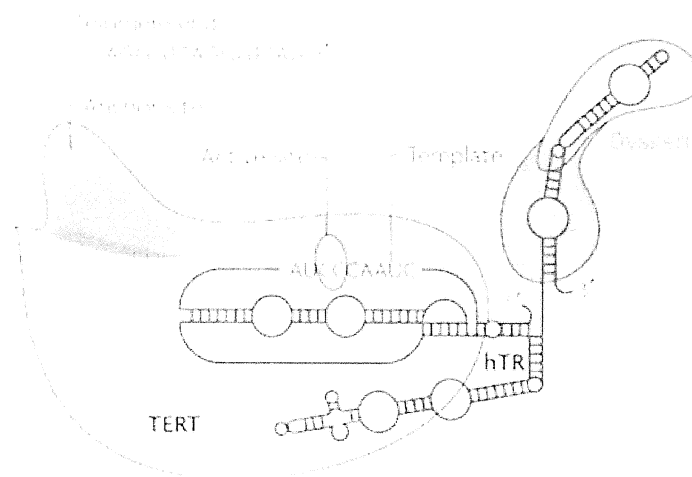


Figure 7: Structure of Telomerase (Harley, 2008). Diagram depicting the telomerase enzyme structure with its two subunits hTERT and hTERC (hTR) as well as the substrate anchor site and active site. The smaller structure corresponds to proteins such as dyskerin that are required for the assembly of the active telomerase enzyme.

The telomerase enzyme is capable of extending the single-stranded telomeric overhang by adding nucleotides to it, thus producing telomeric repeat units anew and opposing the DNA shortening as outlined in the DNA end-replication problem (Chan & Blackburn, 2004). During DNA replication, the extension of the telomeric sequence

involves several steps: Telomerase identifies the hydroxyl (OH) group at the 3' terminus of the telomere and binds to the single-stranded overhang through complementary basepairing with the TERC RNA template region (Mocellin *et al.*, 2013; Chan & Blackburn, 2004). Through the addition of guanine (G) and Thymine (T) nucleotides to the 3' overhang as directed by the RNA template, the 3' chromosomal end is extended. The RNA template is then released from the elongated 3' telomeric terminus, making the latter accessible for further elongation by telomerase or for the polymerase enzyme, which synthesises the complementary cytosine (C) rich lagging-strand by using the extended overhang as its template in turn (Chan & Blackburn, 2004).

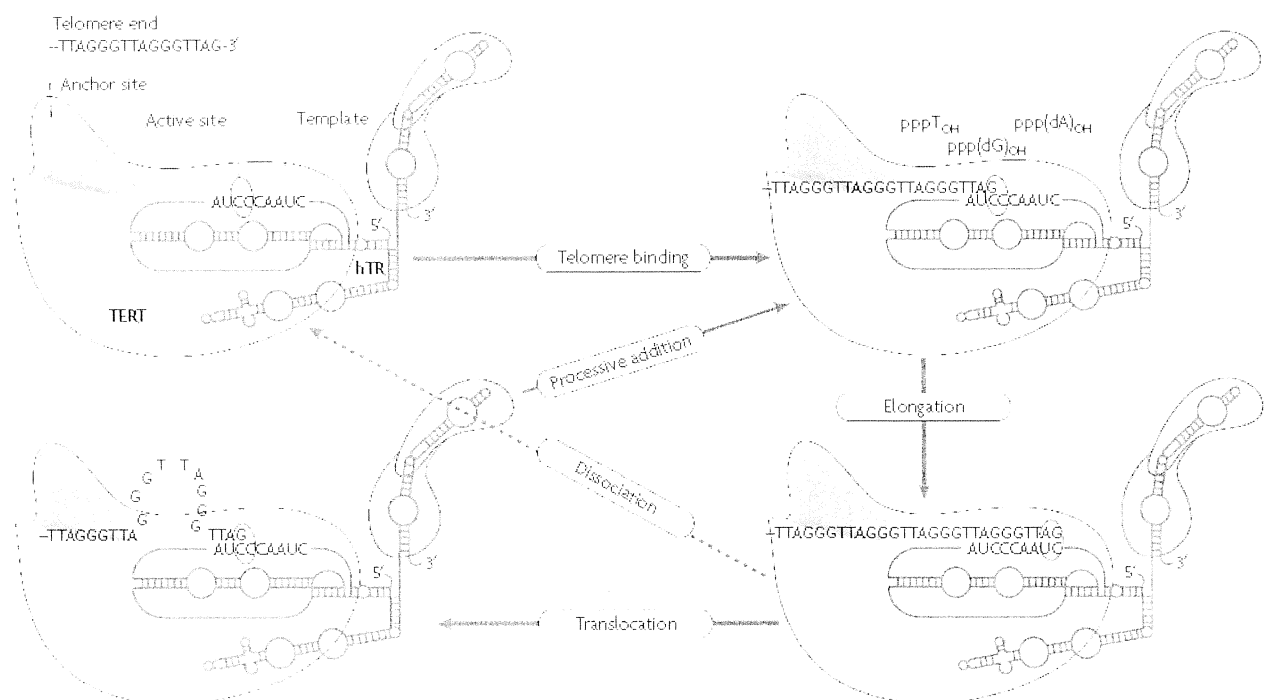


Figure 8: Telomere synthesis by Telomerase (Harley, 2008). Telomere synthesis involves the binding of the TERC template to the 3' single-stranded telomeric overhang, telomere elongation during which the six nucleotides are added to the telomere as directed by the TERC template and translocation, during which the telomerase enzyme repositions for one more round of elongation (processive nucleotide addition) (Harley, 2008). When telomere synthesis is complete, telomerase dissociates from the elongated telomere.

Telomerase expression in cells is regulated at different levels of protein and RNA processing, holoenzyme assembly and is also believed to be regulated by a shelterin negative feedback loop. It is thought that longer telomeres bind more shelterin and are consequently less accessible to telomerase (Buseman *et al.*, 2012). In humans, telomerase is expressed during embryonic development. However, in most adult cells it is repressed with the exception of cells showing ability to regenerate, male germ line cells, stem cells, hematopoietic cells and cells lining the intestine (Cunningham *et al.*, 2006 & Buseman *et al.*, 2012). On the other hand, telomerase also gives most cancer cells the ability to proliferate indefinitely and is expressed in over 85% of cancer cells (Buseman *et al.*, 2012).

With telomerase being active in such a high percentage in cancers, it has meant that it has 'jumped the queue' for targeted cancer therapy.

2. Telomerase Inhibition

The high expression of telomerase in most cancer cells compared to low levels or absence in most adult somatic cells makes telomerase an attractive target in anti-cancer therapy, as there is the chance that a telomerase specific inhibitor will only cause relatively inconsequential side effects (Satyanarayana *et al.*, 2004).

The concept of telomerase inhibition as a possible anti-cancer treatment arose when several experiments showed a correlation between telomerase reactivation and cells' eternal proliferation ability (Blasco & Hahn, 2003). In the absence of telomerase, sufficiently shortened telomeres were found to elicit a DNA damage response, followed by p53 stimulation, which resulted in cellular senescence or

apoptosis (Feldser & Greider, 2007; Herbert *et al.*, 1999; Gonzalez-Suarez *et al.*, 2000). However, overexpression of telomerase was shown to lead to increased spontaneous tumourigenesis (Gonzalez-Suarez *et al.*, 2001; Artandi *et al.*, 2002).

A relevant concern may be the possible effect telomerase inhibition might have on adult somatic cells that normally express telomerase. However, it has been suggested that cancer cells expressing telomerase generally have shorter telomeres compared to somatic cells that are expressing telomerase (Asai, *et al.*, 2003; Marian *et al.*, 2010). Thus, this could offer a therapeutic window during which cancer cells can be induced to undergo apoptosis before normal somatic cells expressing telomerase are negatively affected (Buseman *et al.*, 2012). Additional reduction in tumour mass may be achieved by combining telomerase inhibition with other treatments such as more traditional chemotherapy.

Yeast, mice and human cells that have been engineered *in vitro* to lack telomerase were found not to undergo replicative senescence and apoptosis immediately, but continue to divide if sufficient telomere length is present until the Hayflick limit is reached (Chan & Blackburn, 2004). Therefore, if telomeres are relatively long to start off with, it may take many rounds of cell division before the telomeres have shortened sufficiently, thereby allowing the tumour to establish itself further. However, in more sensitive tests it was shown that the removal of telomerase does have an effect even before telomeres reach their critical short length as it causes hidden telomere damage, e.g. telomeres fused to DNA breaks more often (Chan & Blackburn, 2003). Therefore, it was concluded that telomere length stabilisation is not the only purpose of telomerase and it is thought that the enzyme also functions in tumour cell growth and progression (Blasco & Hahn, 2003).

2.1 Various Approaches Targeting Telomerase

Many different approaches have been investigated to target telomerase, including dominant negative hTERT, ribozymes, reverse transcriptase inhibitors and oligonucleotides. While immunotherapy is an obvious concept in targeted cancer therapy, it does not inhibit telomerase activity. Rather, it activates the immune system to target hTERT peptide fragments that are expressed as antigens specifically on the surface of cancer cells via the Major Histocompatibility Complex (MHC) I pathway (Harley, 2008). However, this review will concentrate on novel treatments targeting and inhibiting telomerase.

2.1.1 Dominant Negative hTERT

A genetic approach for the inhibition of telomerase was explored early on with the modification of conserved amino acids in the hTERT region leading to the expression of so called dominant negative hTERT (DN-hTERT). DN-hTERT has lost its catalytic activity as a result of the mutation and high levels of DN-hTERT expression have therefore been found to lead to telomerase inhibition accompanied by telomere shortening, resulting in replicative senescence and apoptosis (Hahn *et.al*, 1999, Zhang, *et.al*, 1999). The use of DN-hTERT also increased sensitivity of tumour cells to other anti-cancer treatments such as cisplatin (Biroccio *et al.*, 2003; Tentori *et al.*, 2003). However, possible difficulties with this approach include dealing with risks related to gene therapy as well as ensuring efficient uptake into target cancer cells (Olaussen *et al.*, 2006).

2.1.2 Hammerhead Ribozymes

Hammerhead ribozymes, which are antisense RNAs that cleave hTERT mRNA by targeting and hydrolysing specific phosphodiester bonds, were also evaluated and found to cause telomerase inhibition, loss of cell proliferation ability and induction of apoptosis in various cancer cell lines such as breast and ovarian cancer (Ludwig *et al.*, 2001, Saretzki *et al.*, 2001). In spite of this, shortening of telomeres was not always evident and so further evaluation is needed.

2.1.3 Reverse Transcriptase Inhibitors

Since telomerase is a reverse transcriptase enzyme, early inhibition experiments used synthetic nucleoside molecules that inhibit reverse transcriptase enzymes by preventing the integration of DNA nucleotides into the newly created DNA from the RNA template during the reverse transcription process. Once the nucleoside is incorporated into the DNA chain, it prevents binding of subsequent DNA nucleotides and thus preventing chain-termination (Olaussen *et al.*, 2006; DeCian *et al.*, 2008). Examples of such molecules include AZT (3_azido-3_deoxythymidine); even though it was observed to cause some telomere shortening, AZT was not found to be efficient as it only caused a slight reduction in proliferation ability in laboratory tests (Strahl & Blackburn, 1996; Melana *et al.*, 1998).

2.1.4 Oligonucleotides

Many approaches to telomerase inhibition involve small molecules known as oligonucleotides that target hTERT or hTERC. These molecules consist of short segments of nucleic acids of DNA or RNA origin and are usually approximately 13-25 nucleotides long (Dias & Stein, 2002). They have the capability to bind to a specific target sequence by complementary base pairing as introduced by Watson and Crick in 1953. DNA phosphodiester oligonucleotides have not been found to be successful *in vitro* as they are easily degraded by nucleases and the product of this process, dNMP mononucleotide, possibly has cytotoxic effects (Vaenman *et al.*, 1997 cited in Dias & Stein, 2002). Hence, chemical alterations and adaptations have been used to beat these negative side-effects. Peptide nucleic acids (PNAs) consist of a N-(2-aminoethyl) glycine backbone and have been demonstrated to be specific to hTERC regions (Hamilton *et al.*, 1999). While PNAs are resistant to nuclease degradation and bind to RNA with high affinity, they were demonstrated to be poorly distributed *in vivo*, showed low bioavailability and also some toxicity (Rusckovsky, 1997; Hamilton *et al.*, 1999). However, one oligonucleotide, GRN 163L developed by Geron, has shown promising results in *in vitro* and *in vivo* studies and is discussed in the following section.

2.2 GRN 163L - Imetelstat

Much knowledge still needs to be acquired regarding telomerase composition, conformation and recruitment, but it is known that the RNA template region of the enzyme needs to be displayed and accessible for telomere extension (Buseman *et al.*, 2012). Hence, competitive telomerase inhibitors binding to the 11 base template

region of telomerase may be successful in telomerase inhibition. One of those is the short, modified oligonucleotide GRN 163L, also known as Imetelstat, which is the first inhibitor of the telomerase enzyme that is in clinical development (Baerlocher *et al.*, 2013). The nonconjugated parent compound GRN 163 was found to inhibit telomerase, cause telomere shortening and senescence or apoptosis in cancer cells, but lipid conjugation of the compound highly improved its efficacy and potency (Asai *et al.*, 2003; Djojosebroto *et al.*, 2005).

Imetelstat is a lipidated 13-mer oligonucleotide with a N3'-P5' thio-phosphoramidate backbone. In this backbone, the bridging oxygen atoms at the 3' end are substituted by a 3' amine group known as phosphoramidate and sulphur atoms (thio) are also added (Dikmen *et al.*, 2009). These modifications provide increased stability, protect GRN 163L against cellular nuclease activity and increase the molecule's affinity to its target (Dikmen *et al.*, 2009; Geron, 2013).

Imetelstat contains the sequence (TAGGGTTAGACAA) which enables it to target the 13 nucleotides long complementary sequence on hTERC (Buseman *et al.*, 2012). As a result, the telomere cannot bind to the telomerase RNA template and the formation of an active complex with the catalytic subunit hTERT is prevented. It is a potent telomerase inhibitor as shown by the IC₅₀ values for cell-free assays (0.5-10nM) and cell-based assays (0.15-1.77µM) (Baerlocher, *et al.*, 2013). It also shows a long half-life in rodent tissue for 50-90hrs and based on this the half-life in human bone marrow, liver, spleen and tumour is estimated to be 41 hours (Geron, 2013).

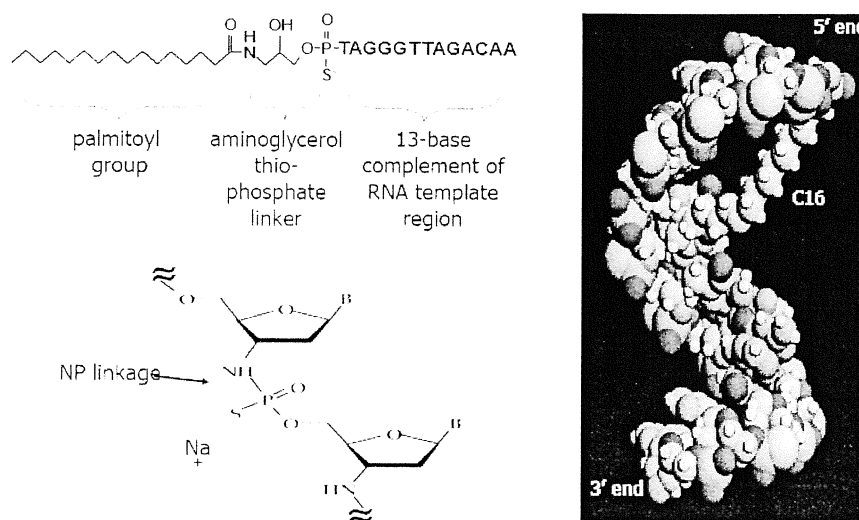


Figure 8: Structure of Imetelstat (Ratain *et al.*, 2010). The simplified chemical structure of the compound can be viewed on the left hand side while its 3 dimensional structure is illustrated in the image on the right.

2.2.1 Preclinical Trials

The effects of GRN 163L were firstly examined in cell lines such as human hepatoma, glioblastoma and lung cells and the compound was found to inhibit telomerase, cause telomere shortening as well as increased rates of anaphase bridges which are characteristic of dysfunctional telomeres, and as a result induced apoptosis (Djojsubroto *et al.*, 2005; Dikmen *et al.*, 2005; Marian *et al.*, 2010).

Additionally, it was shown that treatment with GRN 163L in mouse xenograft models suppressed tumour growth *in vivo*, prevented metastasis and increased sensitivity of tumours to traditional chemotherapy (Asai, *et al.*, 2003; Dikmen *et al.*, 2005; Djojsubroto *et al.*, 2005). Telomerase-independent effects of GRN 163L have also been observed by Mender and colleagues (2013). It is thought to act on the cell's cytoskeleton and reduce the ability of cancerous cells to form colonies and invade.

Imetelstat also showed promise in targeting cancer stem cells, which are defined as cancer cells that have self-renewal potential, initiate tumour formation and regenerate the different cell types that are found in a tumour (Clarke *et al.*, 2006). While conventional therapies do not necessarily target these cancer stem cells that are responsible for cancer reoccurrence, there is evidence that Imetelstat affects cancer stem cells in tumours such as prostate, brain, breast and pancreatic cancers (Marian & Shay, 2009; Marian *et al.*, 2010; Joseph *et al.*, 2010).

Table 1: GRN 163L in pre-clinical trials (Ruden & Puri, 2013)

Tumour type	Effect of treatment
Multiple Myeloma (MM)	Effective inhibition of TA in MM and CSCs; induction of cell death due to apoptosis and significant reduction in tumour cell growth; inhibition of clonogenicity and expression of CSC markers; enhancer myeloma cell death in combination treatment with HSP90 inhibitor 17AGG.
Lung cancer	Dose- and sequence-dependent inhibition of TA; rapid alteration of cell morphology and reduction in cellular attachment and surface spreading; reduction of colony formation and prevention of cell metastases <i>in vitro</i> and <i>in vivo</i> .
Breast cancer	Inhibition of TA; induction of DNA damage response; sensitisation to radiation and chemotherapies; synergistic effect with cisplatin, trastuzumab (HER2* breast cancer cell line), paclitaxel; inhibition of cell migration; reduction of tumour growth, clonogenicity, cell invasion, and metastases; restoration of sensitivity in drug-resistant cells.
Bladder cancer	Significant inhibition of TA; morphological alterations in tumour cells; growth arrest in G ₀ /G ₁ phase.
Glioblastoma (GBM)	Dose-dependent inhibition of TA; reduction in cell proliferation and apoptosis in GBM TICs; ability to cross BBB; preferential accumulation in intracerebral tumours; successful inhibition of tumour growth in intracerebral human tumour xenograft model; enhanced cell survival and activation of DNA damage response pathways in combination with IR and temozolomide treatment.
Prostate cancer	Inhibition of TA in prostate TICs; reduction of TICs; TIC markers and their capacity of self-renewal.
Hepatoma (liver cancer)	Dose-dependent inhibition of TA and tumour cell growth <i>in vitro</i> and <i>in vivo</i> ; reduction of cell proliferation and tumour growth; increased apoptosis and sensitivity due to doxorubicin treatment.
Pancreatic cancer	Inhibition of TA in bulk tumours and TIC subpopulations; reduction in tumour engraftment concomitant with reduction in TIC numbers; inhibition of cell growth and apoptosis with prolonged treatment; reduced tumorigenicity <i>in vivo</i> .
Barrett's adenocarcinoma	Inhibition of TA; induction of senescence or apoptosis; enhanced inhibition of cell proliferation with combination of doxorubicin and ritonavir; significant reduction in tumour size.

TA, telomerase activity; CSCs, cancer stem cells; TICs, tumour-initiating cells; BBB, blood brain barrier

2.2.2 Clinical Trials

The efficacy and good tolerability of Imetelstat in several pre-clinical studies led to the introduction of the compound into a number of stage I and II clinical trials involving different types of cancers such as hematologic tumours as in multiple myeloma and solid tumours as found in breast and non-small cell lung cancer (Ruden and Puri, 2013). In general, the compound was given intravenously and found to have good bioavailability, pharmacokinetics and generally good tolerability (Buseman *et al.*, 2012).

Table 2: GRN 163L in clinical trials (Ruden & Puri, 2013)

Phase	Status	Condition	Drug interventions	Outcome measures	Current results
I	Ongoing	CLD	GRN163L	Safety, tolerability, DLT, MTD, PK, PD	Trial ongoing, no study results yet available
I	Ongoing	Solid tumour malignancies	GRN163L	Safety, DLT, MTD, PK, disease response	31 treated; 4 remain on study; MTD in range 9.4-11.7 mg/kg/d1,d8 of 21 d cy; DLT – thrombocytopenia, myelosuppression and hypersensitivity reactions
I	Ongoing	Multiple myeloma	GRN163L	Safety, MTD, PK, PD, efficacy	DLT – thrombocytopenia & aPTT; MTD in range of 4.8-7.2 mg/kg/2hr IV/t.i.w.
I	Ongoing	Non-small cell lung cancer	GRN163L, paclitaxel (P), carboplatin (C)	Safety, MTD, PK, efficacy	Trial ongoing, no study results available yet
I	Ongoing	Multiple myeloma	GRN163L, bortezomib, dexamethasone	MTD, Safety, PK, efficacy	Trial ongoing, no study results available yet
I/II	Ongoing	Recurrent or metastatic breast cancer	GRN163L, paclitaxel (P), bevacizumab (B)	Safety, MTD, efficacy, PK, efficacy	14 treated; 2 remain on study; dose delays of GRN163L and/or P in later cycles; ORR 38.5%
II	Recruiting	Non-small cell lung cancer	GRN163L, bevacizumab	PFS, ORR, time to all-cause mortality, safety, tolerability	Trial ongoing, no study results available yet
II	Recruiting	Recurrent or metastatic breast cancer	GRN163L, paclitaxel with or without bevacizumab	PFS, ORR, clinical benefit rate, safety, tolerability	Trial ongoing, no study results available yet
II	Recruiting	Essential thrombocythemia	GRN163L, standard of care	Hematologic response, safety, tolerability, number of patients with hematologic toxicities, non-heme grade 3 and 4 AEs and hemorrhagic events	Trial ongoing, no study results available yet
II	Recruiting	Multiple myeloma	GRN163L, standard of care	PFS, ORR, safety, tolerability, number of patients with hematologic toxicities	Trial ongoing, no study results available yet
I	Recruiting	HER2=Breast cancer	GRN163L, trastuzumab	DLT, PK, ORR, PFS, safety and biologic effects of GRN163L in combination with trastuzumab	Trial ongoing, no study results available yet
I	Recruiting	Solid tumours or lymphoma	GRN163L	MTD, toxicities, PK, biologic effects, effect on telomeres and telomerase	Trial ongoing, no study results available yet

DLT, dose limiting toxicity; MTD, maximum tolerated dose; PK, pharmacokinetics, PD, pharmacodynamics; PFS, progression free survival; ORR, objective response rate; AEs, adverse events; aPTT, active thromboplastin time; t.i.w, three times a week

In the phase I trial for locally recurrent or metastatic breast cancer, Imetelstat was administered together with chemotherapy agent paclitaxel and biological therapy agent Bevacizumab (Kozloff *et al.*, 2010). As dose-escalation designs were used to establish Imetelstat's maximum tolerated dose and dose limiting toxicities, many patients faced a reduction or delay in doses of Imetelstat and/or paclitaxel in later cycles due to signs of dose limiting toxicities. Nonetheless, the initial response observed in this trial was positive as over 50% of patients showed a partial or complete response to the treatment. Subsequently, an alternative dosing regimen was also evaluated and found to be a suitable alternative (Ratain *et al.*, 2010). Dose limiting toxicities in phase I trials were established to be thrombocytopenia (low platelet count), neutropenia (low neutrophil count), hypersensitivity and delays in blood clotting (Ratain *et al.*, 2009).

Since the phase I clinical trials showed promising results, four phase II clinical trials were designed. Imetelstat subsequently entered phase II trials for two solid tumour cancers, namely non-small cell lung cancer and advanced breast cancer as well as for two hematologic tumours, namely essential thrombocythemia (ET) and multiple myeloma with GRN163L as the only treatment or in combination with traditional chemotherapy (Geron, 2013). Generally, the dose limiting toxicities observed in phase II trials were thrombocytopenia and neutropenia, but adverse effects were able to be managed and reversed.

Treatment with Imetelstat in a phase II non-small cell lung cancer trial did not initially appear to have a statistically significant effect (Chiappori *et al.*, 2013a). In this trial, Imetelstat was given as a maintenance therapy for non-small cell lung cancer to improve progression-free survival of patients initially treated with chemotherapy. Even though Imetelstat was generally well tolerated and some improvement was

apparent, it was not of statistical significance. However, in *in vitro* studies it was found that sensitivity in cancer cells to Imetelstat improved with shorter telomeres (Chiappori *et al.*, 2013b). Telomere length differs between various tumour types but also between single individuals who carry the same tumour type. Retrospective analysis of the phase II trial showed Imetelstat improved progression-free survival rates in individuals whose tumours were characterised by short telomeres. Further data analysis for overall survival indicated an improvement for individuals whose telomeres were short as well as medium-long. Geron is currently assessing the consequences of these findings on the development of Imetelstat for treatment of solid tumours with short telomeres (Geron, 2013).

Results from phase II trials were particularly positive for patients suffering from ET; all 18 patients treated with Imetelstat showed hematologic responses (Baerlocher *et al.*, 2013). In ET, patients suffer from higher than normal platelet counts and about 50% of ET patients also show molecular mutations such as JAK2 V617F (Geron, 2013). These mutations are thought to arise in cancer stem cells and therefore a molecular response or reduction in JAK2 V617F levels upon treatment with Imetelstat is indicative of the selective inhibitory effect of the compound on the malignant progenitor megakaryocytes that drive the disease. In 16 out of 18 (88.9%) patients, a complete response was observed, which was defined as a reduction in platelet count to normal and maintenance of those levels for at least 4 consecutive weeks (Baerlocher *et al.*, 2013). After achieving a complete response, patients were given an Imetelstat maintenance treatment, during which the dosing was in general reduced with time. The other 2 patients (11.1%) showed a partial response, which was defined as a reduction in platelets count to $\leq 600 \times 10^3/\mu\text{L}$ or 50% platelet reduction maintained for at least 4 weeks. Molecular responses were observed in 7

out of 8 (88%) patients and subsequently maintained in 6 of those. These results suggested that Imetelstat selectively inhibits spontaneous growth of megakaryocytes, the cells in the bone marrow that give rise to platelets, but does not prevent normal growth of megakaryocytes in healthy people. The Imetelstat treatment was generally well tolerated and no discontinuation due to adverse effects was observed (Geron, 2013). Most adverse events were mild-to-moderate in severity and included fatigue, gastrointestinal events and neutropenia. Even though Imetelstat appeared to have a positive treatment effect in patients with ET, medical experts suggested that the already existing therapies for ET are working well for patients and so Geron was advised to investigate the application of Imetelstat in other hematologic cancers such as myelofibrosis (MF) where there is a greater need for additional therapies.

In November 2012, an investigator-sponsored trial was introduced by Dr. Ayalew Tefferi at the Mayo Clinic with the aim to examine safety, efficacy and appropriate dose and dosing regimen of Imetelstat in individuals suffering from MF. While the study is still recruiting patients, over 50 participants are already registered. Two cohorts, A and B, already underwent treatment with Imetelstat. Cohort A consisted of 11 patients who received Imetelstat once every three weeks. Cohort B also consisted of 11 patients, but these received treatment with Imetelstat once every week for four weeks, with subsequent Imetelstat administration once every three weeks. In both cohorts, 2 or more patients met the pre-specified criteria as outlined in the clinical protocol and so both cohorts qualified for further enrolment. Additional enrolment in other cohorts was also initiated to investigate different dosing regimens and to assess the effect of treatment with Imetelstat in various patient populations such as patients with MF that developed into acute myelogenous leukemia.

Preliminary results from trials including cohorts A and B are due to be presented at the 55th American Society of Hematology Annual Meeting and Exposition in New Orleans in December 2013. Geron is hoping to commence a Geron-sponsored, multi-centre trial of Imetelstat in MF in 2014 depending on further data from the investigator-sponsored MF trial as well as information from other experts.

3. Conclusion

This review outlined the importance of telomere length maintenance by the enzyme telomerase in tumorigenesis and subsequently highlighted the promising novel compound Imetelstat, the first telomerase inhibitor to enter clinical development. While the Imetelstat clinical trials are still in their infancy, they appeared to be particularly successful for patients with hematologic tumors as opposed to solid tumors such as breast cancer. It is possible, that this success is due to the route of administration since Imetelstat is generally administered intravenously. Therefore, target sites in the blood may be more accessible than target cells in breast tissue for example. Furthermore, it is not entirely clear if telomerase inhibition may have an effect on normal somatic cells expressing telomerase, such as male germ line cells or stem cells. In telomerase negative mice, whose genes expressing the telomerase subunit TERC or TERT have been silenced, no substantial abnormalities in the phenotype were observed and the mice remained viable and fertile (Blasco *et al.*, 1997; Yuan *et al.*, 1999). However, 4th, 5th and 6th generation telomerase-negative mice demonstrated defects in high proliferation tissues and systems such as the hematopoietic and reproductive system

(Chang, 2005). Defects included a decrease in hematopoietic progenitor cells, a reduction in T and B lymphocyte proliferation and reduced fertility in 4th generation mice leading to sterility in 6th generation mice. While there are differences between the mouse and human model with regards to telomerase (e.g. telomerase is not as tightly regulated in mice), these kinds of experiments may give an indication of possible negative effects resulting from telomerase inhibition. Although telomerase inhibition by Imetelstat was reversible in some *in vitro* studies, longer term treatment may still cause negative side effects (Marian *et al.*, 2010). Even so, some of these may be acceptable for patients who suffer from advanced forms of cancer. Only further clinical trialling and development will demonstrate if telomerase inhibition in general and Imetelstat treatment in particular will be an efficient and relatively safe anti-cancer therapy.

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