*Research areas in cancer cell biology such as cellular senescence, telomerase and cancer stem cells appear to be interrelated and may offer the potential for uncovering novel drug targets with potential to address common cancer phenotypes.*



# Seeding drug discovery: integrating telomerase cancer biology and cellular senescence to uncover new therapeutic opportunities in targeting cancer stem cells

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**Telomerase activation is a hallmark of cancer. Advancement of telomerase as a therapeutic drug target has paved the way for translational opportunities in the related fields of senescence and cancer stem cells. Here, lessons may be learnt that can be applied to drug discovery, particularly with regard to the need to appreciate the relationships between telomerase, senescence and cancer stem cells. When considered as a time line to clinical trial, targeting of telomerase is leading the way to clinical proof-of-concept, with senescence and the cancer stem cell phenotype driving research concepts vital to maintaining a clinical development pipeline.**

## Introduction

Over 10 million new cancer cases will be diagnosed worldwide over the next year, and there is an unmet need for effective, less toxic cancer therapeutics. Our rapidly expanding understanding of how cellular immortality contributes to cancer progression offers an opportunity for the exploitation of the underlying molecular mechanisms into safe and effective new therapies [1–3].

Cancer cell immortality (ulimited replicative capacity) is a cross-cutting theme that draws together three major cancer phenomena of telomerase-mediated telomere maintenance, cellular senescence and cancer stem cell biology [4–7] and as such offers a rich vein of opportunities for the development of cancer therapeutics through mechanism-based approaches to drug design [1–3,8–11]. At present, telomerase represents the best defined and validated target for clinical development within the biology of cellular immortality [1–3,8,11–13] (Figure 1a). Telomerase inhibition in cancer has highlighted how powerful the induction of cellular senescence can be as a tumour-suppressor mechanism [2,6,14]. Thus, progressing knowledge of the molecular pathways regulating senescence will build a network of genes for target discovery. As our understanding of the genes and pathways regulating cellular immortality grows, it is important that these are considered in context of the target cell for neoplastic transformation, and recent progress in the cancer stem cell field have highlighted nascent links to existing knowledge in cellular immortality.

## Telomeres and telomerase: measuring up to the clinic

Telomeres are DNA–protein complexes forming capping structures that stabilise chromosomal ends and prevent them from being recognised as DNA double strand breaks during DNA replication. In humans, telomeric DNA is composed, in the 5′ to 3′ direction, of a hexameric oligonucleotide sequence (TTAGGG) repeated approximately 1000–2000 times. Telomeres are maintained by the telomerase holoenzyme that catalyses the addition of the telomeric repeats on to the ends of chromosomes, thus maintaining their length despite continued cell division. The telomerase holoenzyme functions by utilising an RNA template subunit and a reverse transcriptase unit to catalyse the addition of the telomeric repeats onto the 3′ ends of the eukaryotic chromosome [2,6,14–17].

In adult humans, most normal tissues have low or no detectable telomerase activity. The inactivation of telomerase activity

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1359-6446/06/$ - see front matter  doi:10.1016/j.drudis.2007.06.009

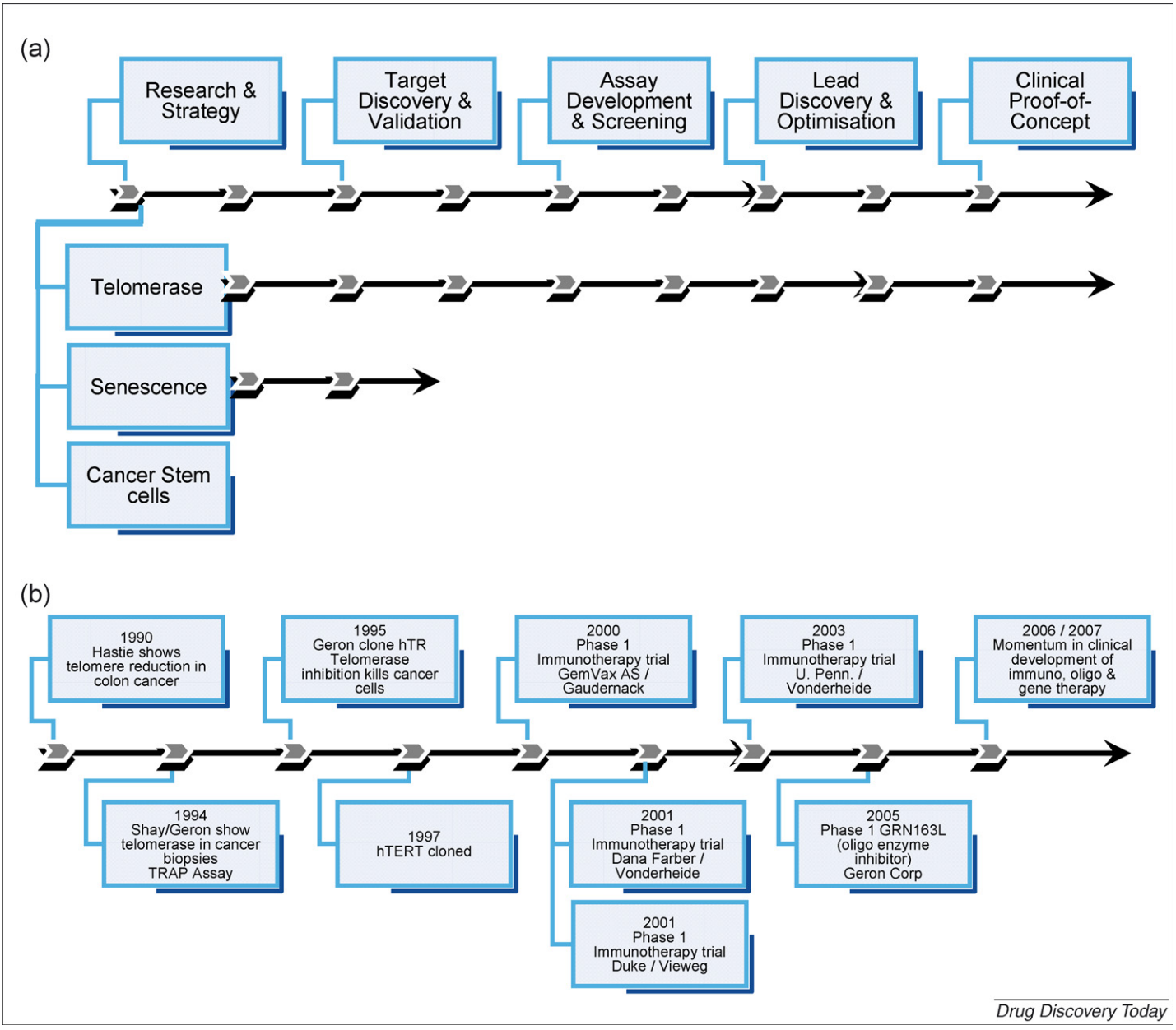


**FIGURE 1**

**(a)** Maintaining a pipeline from research to clinical proof-of-concept. Telomerase has successfully progressed to early clinical trial with senescence and the cancer stem cell phenotype driving research concepts vital to maintaining a clinical development pipeline. Within senescence there are now concerted efforts leading to target discovery and validation with the cancer stem cell field producing valuable concepts with potential to deliver targets in the near future. **(b)** Telomerase cancer timeline highlighting major events ranging from the first demonstration of telomere dysfunction in cancers through the development and application of key assays (TRAP assay) to show high frequency of telomerase activity in human cancers and towards the first series of clinical trials to target telomerase.

together with the loss of telomeric tract during cell division presents a limit to the maximum number of divisions a cell can undertake and therefore a limit to its lifespan, and when cells reach this limit, they enter a state known as senescence. This response to telomere shortening is thought to have evolved as a tumour-suppressive barrier against excessive clonal expansion [2,5,7,18].

In contrast to the situation in normal somatic cells and tissues, telomerase activity is readily detectable in the majority of cancer tissues, including all of the major cancer types such as breast and colon. Telomerase activity is considered to be essential for the continued growth and survival of the malignant cells, so the rationale is that inhibition of this activity will kill the cancerous cells. Since telomerase activity is ubiquitous among a vast range of tumour types, this therapeutics class of drugs has a theoretical application in virtually all human cancers [1–3].

Telomerase drug development strategies can be grouped into four general areas of activity; immunotherapy, gene therapy, oligonucleotide inhibitors and small molecule inhibitors. As yet, traditional small molecule programmes have not yielded candidates suitable for clinical development. This may reflect unique challenges in assay format for high-throughput screening of telomerase inhibitors. Encouragingly, the first proof-of-concept clinical trials using telomerase as a drug target are already underway or recently completed (Figure 1b). Of particular note, the first

**TABLE 1**

**Advantages and disadvantages of current methodologies to detect telomere sequences**

| | *Advantages* | Disadvantages |
|---|---|---|
| **QPCR** | Can analyse many samples (96-well plate);<br>Can be used for blood and cell lines;<br><br>Reproducible but care needed;<br>Sensitive;<br><br>Good correlation with Southern blots. | The result is the mean of a population;<br>Assay variation can result in loss of sensitivity for detecting small telomere length changes as compared with Southern blots;<br>Can be detecting subtelomeric sequence;<br>Results may vary per tissue, for example variability between lymphomas, bone marrow samples and renal cell carcinomas;<br>Formalin fixed/embedded tissues can be problematic. |
| ***In situ* FISH** | Can see spatial distribution of signal within a cell (long and short telomeres);<br>Useful for cryo-fixed clinical samples and sometimes formalin fixed (depending on tissue autofluorescence);<br><br>Possible to combine with antibody labelling to make it cell-type specific (e.g. for cancer cells). | Moderately quantitative (metaphase spreads), but requires care with hybridisation (similar to Southern blot);<br>Can use centromeric probe to standardise between different cell-types, but there is variation from person to person in these sequences, and some probes are better than others (need standardisation);<br>Not high throughput. |
| **Flow FISH** | Can see telomere length across a population (this helps to identify subsets within a population);<br>Pretty easy to set up;<br><br>Good reproducibility;<br><br>Quantitative;<br>Rapid, fairly high throughput;<br>Good correlation with Southern;<br>Commercial assay service lab being established. | Variation observed between individuals when using a centromeric probe;<br>Not yet adapted for solid tumours (not as advanced as assays for haematopoietic cells types);<br>Some success observed with hepatocytes isolated from human livers. |
| **Southern blot** | Widely used and relatively simple to perform;<br>Quantitative;<br>Can be non-radioactive so feasable in clinics;<br>Possible to determine average telomere length and obtain data on distribution;<br>Can detect smaller telomere length changes than with Q-PCR;<br>Good research tool. | Not high throughput;<br>Need approximately 2 million cells minimum;<br>Detection can be biased towards longer telomeres;<br>Probe can hybridise to subtelomeric × region, that is the part that never shortens;<br>Do not get inter-cell variability (unknown if the spread is within or between cells). |
| **STELA** | Can detect very, very short telomeres in non-dividing cells (almost nucleotide resolution);<br>Can measure the amount of telomere loss or gain per cell generation/round of division;<br>Gives good information on distribution;<br>Can give information on clonal analysis. | Do not have probes for all telomeres (only for $n = 7$);<br><br>Considerable start-up challenges in the lab. |

telomerase immunotherapy trials were initiated in 2000 and have gathered considerable momentum since then. Phase I/II trials in lung, breast, melanoma, prostate and pancreas are completed or ongoing with a multicentre phase III trial in pancreatic cancer in combination with chemotherapy due to start soon. In addition, in 2005, the Geron Corporation submitted an investigational new drug application to begin a phase I/II dose-escalation trial of GRN163L, a telomerase targeted oligonucleotide, in patients with CLL. Another phase I trial in patients with solid tumours began in 2006. Both trials are dose-escalation studies designed primarily to evaluate the safety, tolerability and pharmacokinetics of intravenous GRN163L infusion. Success with complex biotherapeutics such as immunotherapy and oligonucleotode inhibitors will deliver proof-of-concept that telomerase is a valid target for drug development [3,8].

The breadth of drug development programmes aimed at telomere biology has been sustained through the iterative approach of assay and target validation, and this is a paradigm for drug development for complex biological targets [1,19,20]. Now that a variety of approaches targeting telomerase have reached the clinic or are entering clinical development, the need for robust biomarkers and assays, pharmacodynamic endpoints and prognostic markers is of a high priority [1,21–23], as these will assist future rational drug development programmes centred around telomerase cancer biology.

An array of telomere length and telomerase assays is available (see Tables 1 and 2). Selection of the most appropriate assay for a particular application is key to acquiring quality data. The perceived advantages and disadvantages of each assay are listed in Tables 1 and 2.

### *Recommendations and opportunities*

The development of both universal and specific standards for telomere length assays and telomerase assays is a particular challenge. Standardisation would benefit the research community and enable the expansion of these potentially valuable assays into clinical and commercial applications. Development of standard reference samples for both telomere length and telomerase assays

**TABLE 2**

**Advantages and disadvantages of current methodologies to detect telomerase activity**

| | *Advantages* | Disadvantages |
|---|---|---|
| **Primer Extension Assay** | Can isolate extracts with high specific activity for HTS of drug compounds;<br>Can avoid drug compounds interfering with the PCR;<br>Higher throughput than PCR. | Amount of enzyme required (cost);<br>Large cell volumes may be required, for example if using say 10 000 L;<br>If purity is low then kinetics may be difficult;<br>Recombinant enzyme (rather than extracts) can be used but reconstitution can be inefficient. |
| **TRAP** | Simple and easy to follow;<br>Kit-based;<br>Can buy 'off the shelf';<br>Possible to assay small numbers of cells; Reproducible;<br>Semi-quantitative (with correct standards, careful controls and repetition);<br>Can be just a yes/no answer;<br>Good visualisation from gels. | Data are the sample average;<br>With any PCR-based assay, 1% of sample might be giving 80% of signal);<br>Kit is expensive;<br>Reproducibility is an issue if used rarely;<br>Not trusted as a clinical tool because of lack of reproducibility (may depend on sample integrity/tissue preservation);<br>Sample components (e.g. haem) can inhibit Taq polymerase. |
| **RT QPCR** | Quantitative;<br>Quantification does not depend on the numbers you get from a single total PCR cycle (internal standardisation);<br>Good for scale-up to multi-well dishes. | Difficulties in drug discovery need to pre-screen for primer effects, quadroplex binding and so on;<br>Lack of visualisation can be a problem for some people;<br>Visualisation of a ladder on a gel can make quantitation difficult. |

is urgently required. To more accurately address research questions, ease of use and technology transfer of telomere and telomerase assays are desirable, including the more challenging assays such as metaphase FISH. In addition, matching what happens in *in vitro* biochemical assays to whole cells and tissues is a particular challenge. For example, it is essential to establish how much *in vitro* telomerase measurements are reflective of the *in vivo* cancer tissue.

Opportunities exist for the development of specific and sensitive antibodies to enable analysis of individual cells and the study of intra-sample variability and tissue variability. Such assays could be categorised as complementary to existing techniques.

A noticeable trend in telomere length assays is the shift from the Southern blot as the 'tried and true' to QPCR for high-throughput assays (Tables 1 and 2). Opportunities exist for the development of high-throughput *in situ* FISH, Flow FISH, telomere assays for solid tumours and for assays of telomere function and dysfunction (e.g. dynamics of repair). For telomerase assays, the impact of telomere-binding proteins such as those shown in Figure 2 requires to be clarified.

Telomerase activity and telomere maintenance are mediated by a number of genes (Figure 2). The complex 'biological map' of intra-cellular interactions that control telomerase activity and telomere maintenance is not yet completely defined, but progress has been rapid and encouraging. It is important to move away from considering telomerase as a single molecular target: telomeres, telomerase and the pathways regulating telomerase activity and offer a wealth of targets for drug development and, as our understanding of telomerase biology grows, so does the number of potential therapeutic strategies [1–3,8]. However, telomerase may just be the start of the discovery process. Given the importance of telomerase in preventing cellular senescence and since inhibition of telomerase in cancer cells results in cellular senescence, resolution of the molecular pathways controlling senescence (Figure 3) may also offer further opportunities for drug development [18,24].

## Senescence

Senescence is an irreversible arrest of cell proliferation. Senescent cells remain viable and metabolically active but cannot re-initiate DNA replication in response to mitogenic stimuli and futhermore may become resistant to apoptosis. Senescence is also associated with altered cellular morphology and size and altered expression of genes involved in many aspects of cell physiology [6,14,18,24]. Senescence depends on a number of signalling pathways that together result in a permanent and irreversible cell cycle blockade (Figure 3).

Cellular senescence provides a barrier to excessive cellular growth and is triggered by four main mechanisms (Table 3) [6,9,10,18,24–27]. Telomere attrition during extended cell proliferation will eventually lead to cellular senescence as critically short telomeres will be recognised as DNA damage, thereby triggering growth arrest. Whilst this proliferative barrier provides a limit to the outgrowth of a cell, senescence can also be triggered in response to drug exposure, cellular stress and oncogenic signalling. As senescent cells do not proliferate, it has been proposed that cellular senescence is a major barrier to cancerous transformation and premature induction of cellular senescence can therefore be targeted for anti-cancer therapy in tumourigenic cells (Figure 4).

A crucial issue in developing a drug discovery programme is understanding how senescence is regulated at the molecular level and importantly, which cell populations are susceptible to senescence. Our current understanding of this is still fragmentary.

### *Is induction of senescence **in vivo** a desirable clinical outcome?*

Many current cancer therapies kill cancer cells by inducing programmed cell death, or apoptosis (Figure 4). However, treatment of

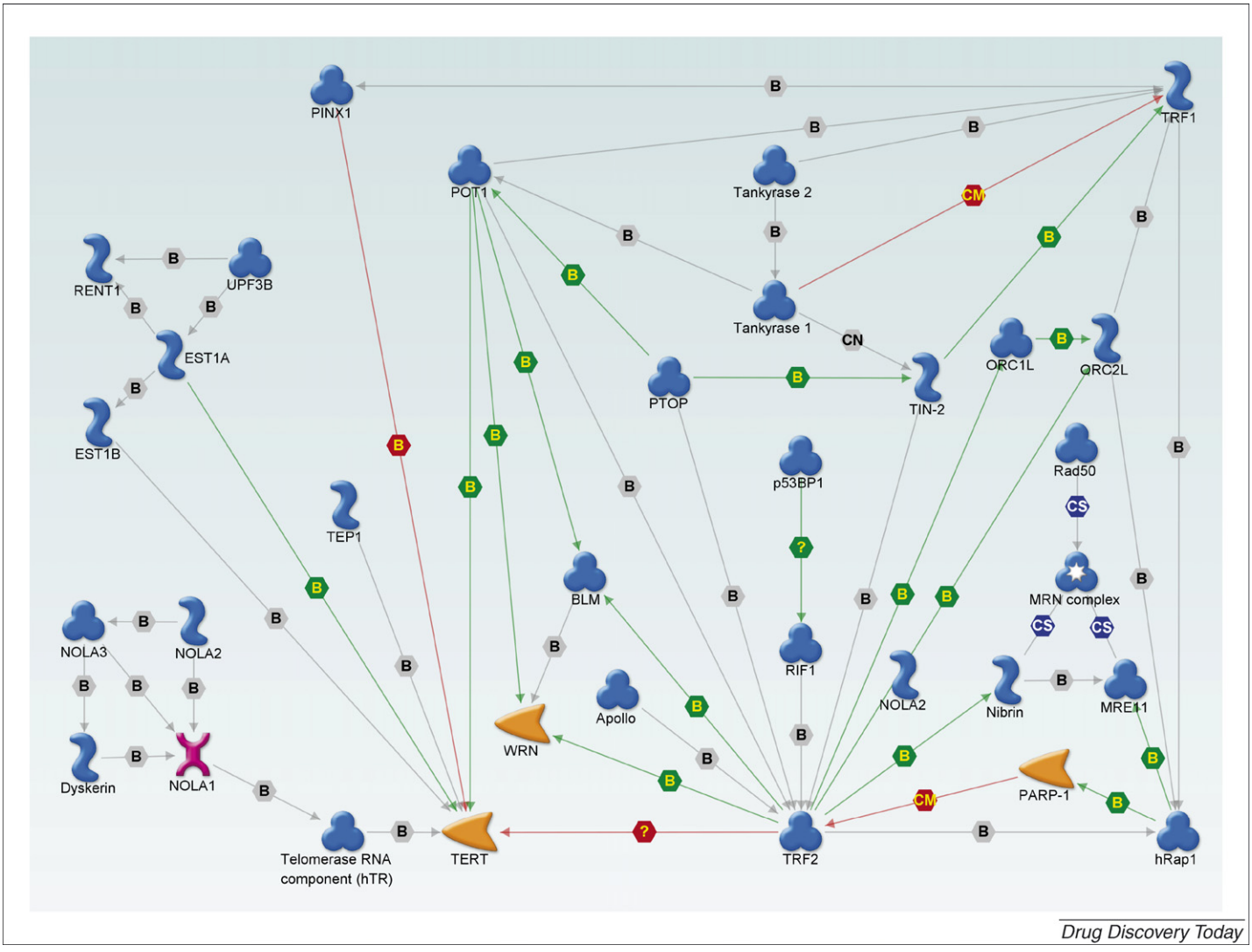


**FIGURE 2**

Examples of genes and pathways involved in telomere maintenance. Telomere structure is maintained through the interaction of a number of interrelated gene products and protein complexes including telomerase (hTR and hTERT) and the Shelterin complex (including TRF2, TIN2, Tankyrase and POT1). In addition, there are alternative mechanisms to telomerase (ALT mechanism, including the MRN complex). Key to network objects. Red arrows: inhibitory effects. Green arrows: activation. Grey arrows: unknown effect of interaction. P: phosphorylaton event. B: binding event. CN: competition. TR: transcriptional repression. IE: influence on expression. CM: covalent modifications. CS: complex subunit. Pathway analysis was carried out using Metacore MapEditor (GeneGo Inc.: http://www.genego.com/). Details on individual genes can be found at the GeneCards web site: http://www.genecards.org/index.shtml.

tumours in animal models with telomerase inhibitors can result in an inhibition of tumour growth strongly suggesting that activation of senescence pathways may have therapeutic values [23,28,29]. Interestingly, several chemotherapy drugs, such as adriamycin and cisplatin, and also radiation can induce a senescence-like phenotype (SLP) in cancer cell lines *in vitro* [18,24,30,31]. Induction of SLP is a major response of tumour cells in culture to cytotoxic drugs and is the most prominent marker of growth arrest induced by cytotoxic drugs, although it is not known if these agents can induce SLP in human cancers during therapy. The major gap in this field is an understanding of the role of senescence *in vivo* in human tumours and its induction by therapy.

Translating these cell-based observations into a drug discovery strategy will determine whether induction of senescence can be exploited as a therapeutic endpoint and will have implications for the potential of telomerase inhibitors as well as current drugs in the management of malignant disease.

To explore these opportunities, researchers first need to develop robust methods of actually measuring the senescence phenotype *in vivo*.

### *How can senescence be detected?*

There are currently a number of commonly used biomarkers for the detection of senescence *in vivo* or in clinical samples (see Box 1). However, these are at best associated markers that are also linked to other cellular processes such as differentiation and cell cycle control. In addition, it is unclear whether they can distinguish between the different routes to senescence (Table 3). Recently, cell biology techniques and array screening have identified putative markers and underlying biological pathways and genes (Figure 3) Taken together, a panel of assays can be used to determine senescence

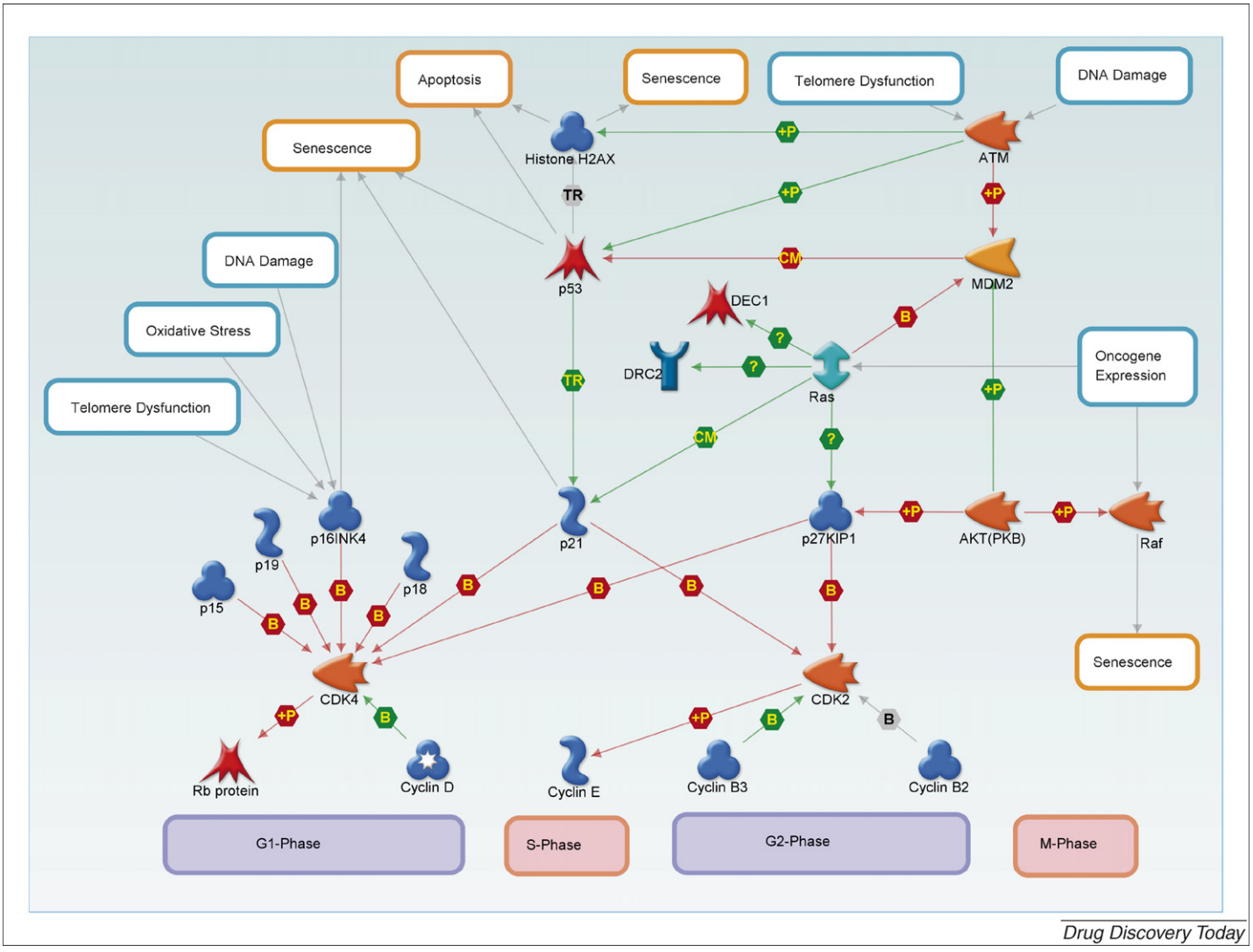


**FIGURE 3**

Examples of genes and pathways involved in cellular senescence. A number of genes are now associated with cellular senescence including p21, p27 and p16. Possible relationships between senescence associated genes, and cellular processes such as telomere dysfunction, DNA damage, stress and oncogene expression and cell cycle progression are shown. Key to network objects as for Figure 2.

(Box 1 and Figure 3), but there is room for improvement. However, the emerging gene signatures are proving to be of value, and it is now important to distinguish between indicators of senescence and causative gene products [9,24,26,32].

### *What are the translational opportunities?*

Although significant research funding is dedicated to the study of apoptosis (as the traditional objective of anti-cancer therapies), there is a paucity of data regarding the induction and clinical relevance of senescence. Fundamental questions about the biology of senescence remain to be answered; for example, it is not known whether senescent cells can be 'reactivated' into proliferation, a situation to be avoided in cancer therapy. Treatment-induced senescence (SLP) appears to be relevant across a range of tumour types but this is yet to be rigorously tested [9,18,24,26,27,32].

As demonstrated by the telomerase inhibitor studies, there is a need for both good animal models and human data to address the questions of whether or not induction or avoidance of senescence

**TABLE 3**

**Routes to cellular senescence**

| Senescence route | Distinguishing features |
|---|---|
| Telomere-dependent replicative senescence | Progressive telomere shortening with cell division |
| Culture-shock-induced senescence | Extrinsic signals/stresses that cells experience when they are explanted into culture |
| Oncogene-induced senescence | Aberrant proliferative signals from oncogenes |
| Drug-induced senescence-like phenotype | Terminal growth arrest after drug exposure |

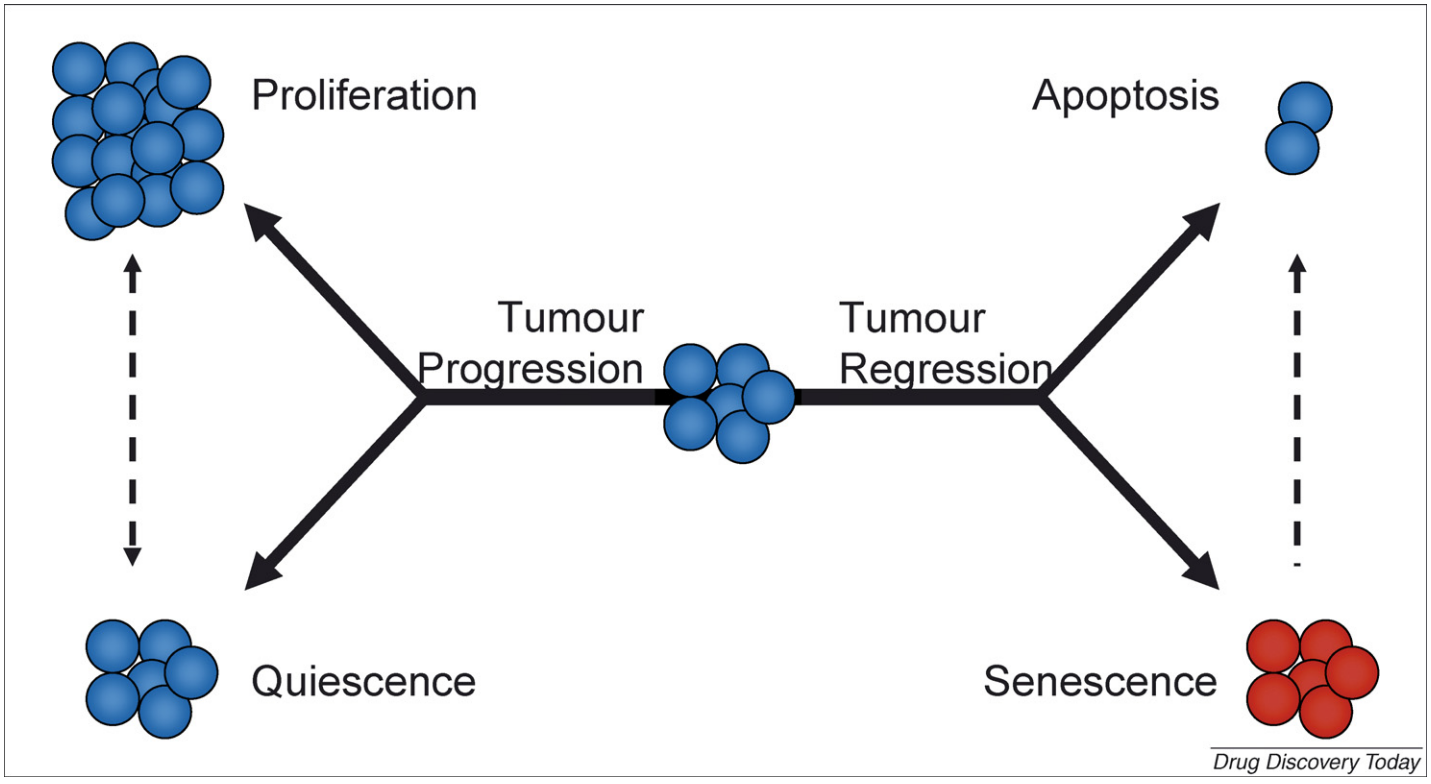


**FIGURE 4**

Dynamics of tumour growth in response to therapy. In the absence of therapy, tumour progression will occur and cells may move between quiescent and proliferative states. The primary aim of existing anti-cancer therapeutics is to induce apoptosis. However, inducing senescence may also be a route to tumour regression, and there is a need for greater understanding of the molecular basis for the senescent phenotype.

in patients is indeed a desirable clinical outcome. Assays are required to enable clinical studies to identify correlations between phenotype and outcome. Such assays would be valuable prognostic tools to aid clinical decision making when patients' tumours appear to become resistant to therapy. Looking into the future, imaging is making a major impact on clinical management. Senescent cells by definition do not proliferate but do remain metabolically active. This leads to the possibility that, should their cell metabolism differ from that in normal cells, imaging modalities such as Positron Emission Spectroscopy (PET) may be used to assess levels of metabolic activity and experimentally available tracers may facilitate differential identification of senescent and non-senescent cells in patients.

In terms of drug discovery, components of the various senescence pathways represent potential targets of the future, pending a better understanding of the components and interaction of these biological pathways. As exemplified in Figure 3 this effort is well underway with an emerging network of genes linked to senescence. 'Proof of concept' and target validation are necessary before senescence will be seriously considered as an area for widespread commercial drug discovery [19,33,34]. However, the Geron Corporation and Senex Biotechnology already have active programmes on telomerase and cyclin-dependent kinase inhibitor (CDKI) proteins.

Now that we are accruing extensive lists of genes and pathways associated with senescence and immortalisation (Figures 2 and 3), it is important these are viewed in context of rapidly emerging concepts in the cell biology of cancer most notably, the cancer stem cell. This will ensure concurrence of the molecular target for drug discovery with the correct cellular target.

**BOX 1**

**Markers of senescence**

| |
|---|
| Morphology |
| Senescence-associated heterochromatic foci (SAHF) |
| Senescence-associated beta galactosidase activity |
| Gene expression signature (p21 and p16 increases) |
| DNA damage response (H2AX) |

## Cancer stem cells

The investigation of the target cell for neoplastic transformation and the molecular mechanisms by which this cell has subverted normal proliferative signals is crucial for the development of novel therapeutic strategies. The identification of cancer stem cells (or tumour initiating cells) suggests that the bulk of a tumour may be derived and maintained from this rare cell but that current therapies may not be eradicating the cancer stem cell population (Figure 5). Thus, novel strategies designed to specifically eradicate the cancer stem cell whilst sparing the normal cell population may be required [4,7,35–39].

In the context of drug development, cancer stem cells are at present in a very early stage (Figure 1a). The existence of cancer stem cells is presently a hypothesis, and sceptics argue that the

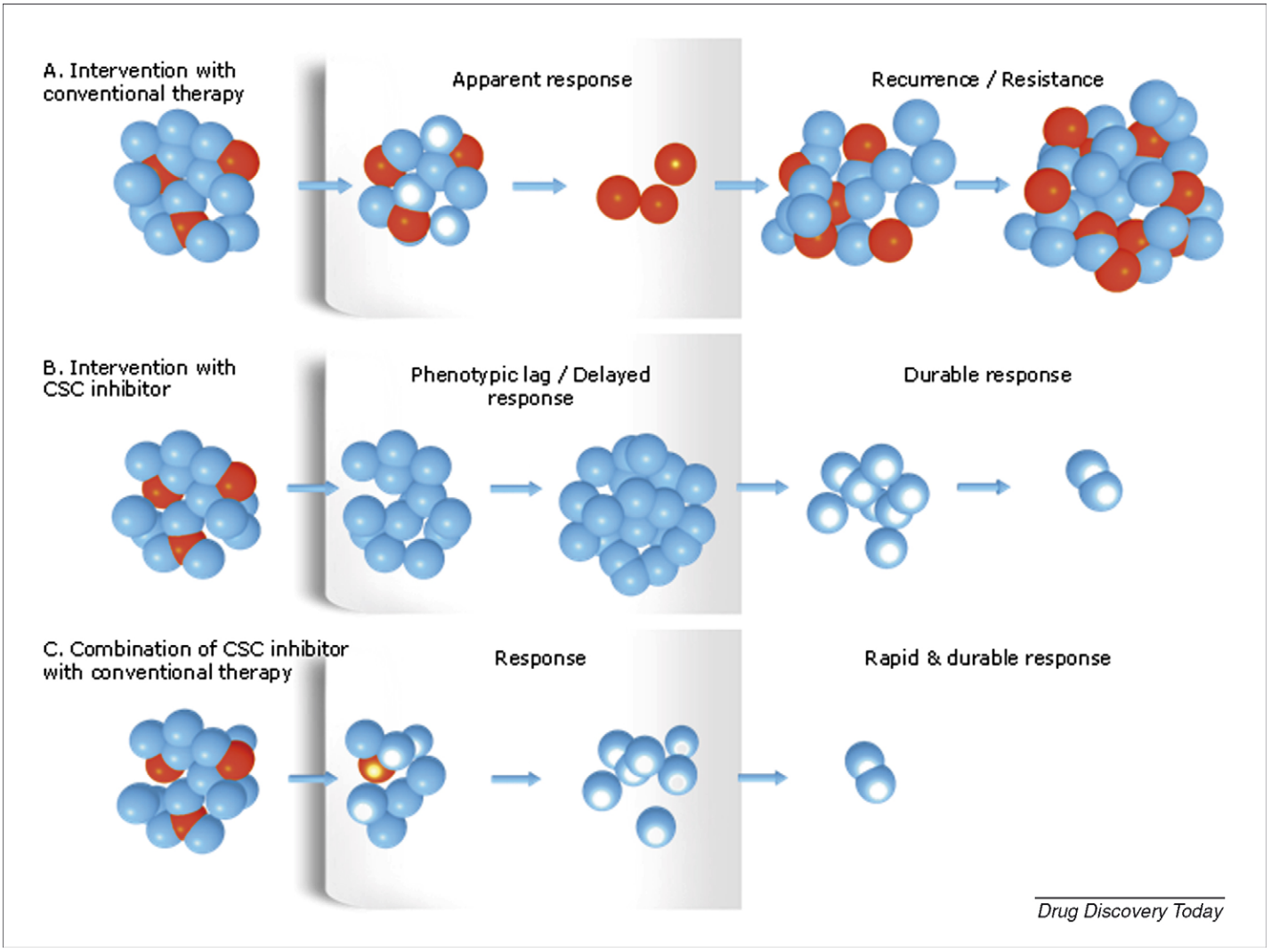


**FIGURE 5**

A model for cancer stem cells (CSC) in tumour progression. A tumour may initially respond to conventional therapies resulting in an apparent response but in many cases the cancer recurs **(A)**. The presence of a rare cancer stem cell population that is refractory to conventional therapy and can therefore initiate re-growth of the tumour is an attractive hypothesis. Indeed, should this same cell population display a drug resistance phenotype, then an increase in its prevalence over time may also explain in part the development of drug resistance. Intervention with a specific inhibitor that targets the CSC **(B)** would remove the tumour initiating cells. However, there may be a delay between removing the CSC population and observation of a reduction in tumour mass. For a rapid and durable response **(C)**, a combination approach using both the CSC inhibitor and conventional therapy may be most effective. CSCs are depicted in orange.

burden of proof remains; this is because of the lack of specific markers and appropriate assays and the difficulty of propagating putative cancer stem cells. However, the study of cancer stem cells is gaining momentum as this concept offers a plausible explanation for cancer recurrence, metastasis and drug resistance (Figure 5) [4,38,39]. Acceptance of the hypothesis is becoming more widespread as a body of supporting evidence emerges (see Box 2).

Originally identified in haematopoietic cancers and in animal models, cancer stem cells have now been putatively identified in common cancer phenotypes including breast, prostate, colon, brain and pancreas [4,36–43].

### *Definition of cancer stem cells*

By definition cancer stem cells have characteristics similar to adult tissue stem cells, in particular the ability to self-renew. As yet there are little data as to what distinguishes a cancer stem cell from a normal stem cell. Several lines of evidence support the existence of cancer stem cells including the presence of small subpopulations of tumour cells with the ability to initiate further tumour formation [4,39,44].

### *How can cancer stem cells be detected?*

Detection and purification of cancer stem cells has posed a severe technical challenge. A scarcity of markers and functional assays coupled with the inability to propagate cancer stem cells *in vitro* renders analysis difficult until the advent of non-destructive tissue fractionation, based on cell surface antigenic profile and cellular adhesive properties [4,39,44]. It remains likely that cancer stem cells from different tumours and from different tissue types will express different marker profiles, but there may be a commonality of 'stemness antigens'.

Although evidence supports the existence of cancer stem cells in solid tumours such as prostate and brain, the concept of cancer

**BOX 2**

**Assumptions and supporting evidence for the cancer stem cell hypothesis of cancer development**

- Stem cells are present in adult tissues
- Adult tissue stem cells can be targets for carcinogenesis and transformation
- Malignant potential may be related to the stem cell potential of the target cell for carcinogenesis
- The self-renewal capacity of stem cells parallels that of the cancer cell
- Mutations in pathways disturbing proliferative life span, cell cycle and differentiation may be required to reveal full malignant potential of the cancer stem cell
- Cancer stem cells are rare within the tumour mass and are necessary to sustain tumour growth

stem cells is considerably more advanced for haematological cancers [40]. Techniques are now generally available to facilitate the enrichment of subpopulations in haematological cancer, which provide a more easily accessible basis for study. Definitive proof of distinct cancer stem cell populations is required. This represents a major opportunity for researchers and for drug discovery.

*How are cancer stem cells relevant to cancer therapy?*

Cancer stem cells are a possible explanation of recurrent disease, metastasis and drug resistance in cancer (Figure 5). Whilst a tumour may initially respond to conventional therapies resulting in an apparent response, in many cases the cancer recurs. The presence of a rare cancer stem cell population that is refractory to conventional therapy and can therefore initiate re-growth of the tumour is an attractive hypothesis. Indeed, should this same cell population display a drug resistance phenotype, then an increase in its prevalence over time may also explain partly the development of drug resistance (Figure 5) [4,36,38–40,44]. The existence of a stem-like fraction of cells in the Hoechst 33342 dye effluxing 'side population' on FACS analysis of some tumours (and indeed normal tissues) argues strongly in favour of the expression of ABC multidrug resistance genes in stem cell populations.

*Translational opportunities*

If proven, the cancer stem cell hypothesis would open up opportunities for identifying new drug targets through gene and protein profiling approaches. This would potentially result in therapeutics that could address deficits of current cancer therapeutics such as recurrence, metastasis and drug resistance.

To fully explore the clinical relevance of cancer stem cells, the cancer stem cell hypothesis first needs to be validated. To this end, development of tools that enable cancer stem cells to be propagated and maintained as a pure population in culture should be of particular priority as sufficient numbers of cells will be required for molecular profiling and functional assays including cell-based compound screens [3,11,45]. In parallel, techniques, assays and markers that facilitate characterisation of cancer stem cells require development. Together, this knowledge could then be used to target cancer stem cells for elimination (Figure 5) [4,36,38–40,44].

Whilst most investigations to date have focused primarily on defining cancer stem cells through cell surface markers and self-renewal assays both in tissue culture and animal tumour models, there are intriguing insights beginning to emerge from small scale gene and protein expression studies on these cells. A common theme appears to be the presence of signalling pathways often associated with normal stem cell populations and involved in self-renewal but which can become altered in cancer cells such as SHH, Notch, WNT and BMI [4,36–42,44–47]. Thus, it may be possible to fast track the drug discovery process by developing research programmes around known pathways with plausible links to the cancer stem cell.

In this regard, the presence of telomerase activity in breast cancer stem cells highlights a significant potential for telomerase therapeutics [48]. Given that at least some adult tissue stem cells are telomerase negative, telomerase is a possible target worthy of attention in the cancer stem cell field [49–52]. Elucidation of cancer stem cell biology is likely to occur in parallel with the development of knowledge of cell biology in the normal stem cell field. Clearly, current molecular biology and omic technologies will also play an important role in distinguishing the cancer stem cell, the normal stem cell and the daughter population. However, high and inducible levels of expression of differentiation-associated genes in the bulk population of cells in a tumour (relative to the stem cell fraction) place particular emphasis on tools to achieve homogeneous populations, if indeed they exist. Such profiling will enable discovery and validation of novel drug targets. Potential drug molecules will need to be rigorously tested against normal stem cell populations to guard against the development of a 'general stem cell poison'; this is expected to be particularly challenging. Catalogues of gene expression profiles descriptive of stem and progenitor cells from a range of normal tissue stem cells are now beginning to appear in public databases.

Looking ahead to the development of therapeutics, traditional approaches to drug discovery have often relied on short-term studies with cell lines looking at endpoints such as cell kill. For developing drugs that address cancer stem cells, longer term studies are needed as there may be a 'phenotypic lag' between administration of the therapeutic and an observable response (Figure 5) and is applicable both to assays screening for cancer stem cell active compounds and within subsequent clinical use. This is a situation familiar to those working in the development of telomerase inhibitors, and indeed other areas such as anti-angiogenic therapies, and does not represent a major issue [2,5,14].

**BOX 3**

**Ongoing questions and cancer stem cell biology**

| |
|---|
| Are cancer stem cells present in all cancer types? |
| Are there fundamental differences between solid tumour and haemopoetic cancers? |
| Do cancer stem cells exist as a homogeneous or heterogeneous population? |
| Is telomerase expression part of the immortality phenotype of normal and cancer stem cells? |
| Are some tumours composed only of cancer stem cells? |
| How can we develop therapeutics that target cancer stem cells? |

Looking further ahead to the clinical development of such therapeutics, the goal must be a durable clinical response and not necessarily an immediate complete response or complete remission. This will impact on the cost, duration and complexity of clinical studies. Combination therapies simultaneously targeting the cancer stem cell population and the bulk population could make it possible to have a complete response in conjunction with a durable response (Figure 5).

In conclusion, more knowledge is key. Translational opportunities are dependent on a better understanding of the molecular and cellular pathways that underlie the cancer stem cell phenotype. More needs to be known about cancer stem cell biology and about normal stem cell biology including senescence pathways and cellular immortality [7,35,49,53,54] (see Box 3).

## Conclusions

Telomerase activity and senescence offer a natural partnership for therapeutic development. Indeed, many telomerase-based therapeutics result in a senescence response. Understanding the interface between telomerase, telomere dysfunction and the senescent phenotype will add value to targeting telomerase and provide additional targets for intervention, biomarkers for patient selection and prognosis. Targeting senescence offers potential targets for future cancer therapies complementary to existing interventions aimed at apoptosis (Figure 2). Our understanding of cellular senescence is maturing rapidly with a sound body of research beginning to seed drug discovery programmes (Figure 1a).

There are clear synergies between senescence, telomerase and the cancer stem cell, yet at present the cancer stem cell field is still relatively young. Drug resistance, recurrence, residual disease and metastasis remain issues for current cancer therapies. The cancer stem cell hypothesis offers an explanation and potential therapeutic target to address these issues. Ideas are not in short supply and with additional opinion, technologies and knowledge from neighbouring fields such as telomerase and cellular senescence, clearly defined targets will emerge from the research base in the near future (Figure 1a).

## Acknowledgements

A British Association of Cancer Research (BACR) Special Conference entitled 'Telomerase & Cancer Stem Cells: Exploiting Cellular Immortality for Therapeutic Gain' was held in York, UK on 4–6 September 2006. During this meeting, three open surgery sessions were conducted to ascertain current opinion on the topics of Senescence, Cancer Stem Cells and Telomeres and Telomerase. The audience, which totalled 60, and mostly from Europe and the USA, comprised of key opinion leaders in each of the three related topic areas. The content of this report is based on this information and reported by the authors on behalf of the meeting. A number of participants are supported by Cancer Research UK and European Community grant LSHC-CT-2004-502943.

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