

Oncogene addiction as a foundational rationale for targeted anti-cancer therapy: promises and perils

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Introduction

Coordinated efforts by the International Cancer Genome Consortium and other Institutions have started to reveal the complex and heterogeneous 'genomic landscapes' of many human cancer types with unprecedented accuracy (Hudson et al, 2010; Stratton et al, 2009). A variegated repertoire of genetic aberrations – including point mutations, small insertions/deletions, gene amplifications and chromosomal rearrangements – leads to a 'picture' in which recurrent lesions in dominantly acting, driver oncogenes and tumour suppressor genes emerge as 'peaks' over a flatter line of infrequently occurring, passenger mutations (the 'hills'; Pleasance et al, 2010; Velculescu, 2008; Wood et al, 2007). The latter probably represent non-adaptive – yet potentially fitness-increasing – mutations in genes involved in widely different cellular functions, acquired at a certain point during tumour evolution. This picture has supported the classic notion of cancer as 'a hundred of diseases', raising both hopes and concerns: while discovery strategies aimed to the identification of novel driver oncogenic lesions are likely to enrich the

A decade has elapsed since the concept of oncogene addiction was first proposed. It postulates that – despite the diverse array of genetic lesions typical of cancer – some tumours rely on one single dominant oncogene for growth and survival, so that inhibition of this specific oncogene is sufficient to halt the neoplastic phenotype. A large amount of evidence has proven the pervasive power of this notion, both in basic research and in therapeutic applications. However, in the face of such a considerable body of knowledge, the intimate molecular mechanisms mediating this phenomenon remain elusive. At the clinical level, successful translation of the oncogene addiction model into the rational and effective design of targeted therapeutics against individual oncoproteins still faces major obstacles, mainly due to the emergence of escape mechanisms and drug resistance. Here, we offer an overview of the relevant literature, encompassing both biological aspects and recent clinical insights. We discuss the key advantages and pitfalls of this concept and reconsider it as an illustrative principle to guide post-genomic cancer research and drug development.

catalogue of therapeutic targets, the task of developing therapies suitable for successful treatment of the full spectrum of cancers, with all their intrinsic heterogeneity, can be easily seen as Herculean and almost impossible (Chin et al, 2011; Chin and Gray, 2008). Yet, the success of therapies that target specific oncoproteins in molecularly defined subsets of cancer patients has provided formal and practical demonstration that, at least in some tumour types, abrogation of the function of individual oncogenic products is sufficient to induce tumour regression or stabilization. Hence, it appears that not all peaks within a tumour have been created equal, and many can be overshadowed by one (Haber et al, 2011; Macconail and Garraway, 2010).

As often, the reversal of the cancerous phenotype induced by the therapy observed in the clinic was predicted by mechanistic studies in the laboratory. Using both *in vitro* and *in vivo* models

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Table 1. Experimental examples of oncogene addiction

Oncogene	Models		
	<i>In vitro</i>	<i>In vivo</i>	References
<i>MYC</i>	RNAi in haematopoietic cells	Conditional <i>MYC</i> -driven mouse models of leukaemia	Felsher and Bishop (1999), Loke et al (1988)
<i>H-RAS, K-RAS</i>	Farnesyl transferase inhibitors in <i>H-RAS</i> -transformed and <i>K-RAS</i> ^{mut} expressing cell lines	Conditional <i>K-RAS</i> ^{mut} -driven mouse model of lung cancer	Fisher et al (2001), Kohl et al (1994)
<i>ABL</i>	Small-molecule inhibitor in <i>BCR-ABL</i> expressing cell lines	Systemic antisense oligodeoxynucleotides delivery in mouse model of CML	Druker et al (1996), Skorski et al (1994)
<i>HER2</i>	RNAi in <i>erbB-2</i> amplified breast carcinoma cell lines	Conditional <i>erbB-2</i> -driven mouse model of mammary cancer	Colomer et al (1994), Moody et al (2002)
<i>EGFR</i>	Ribozyme against aberrant <i>EGFR</i> -expressing cell line	mAb in <i>EGFR</i> -amplified xenografts	Luwor et al (2001), Yamazaki et al (1998)

RNAi, RNA interference; mAb, monoclonal antibody.

of gain-of-function tumourigenesis, it was found that acute inactivation of transforming oncogenes leads to cell-cycle arrest, differentiation or apoptosis, depending on tissue contexts (Table 1; reviewed in Sharma and Settleman, 2007; Weinstein and Joe, 2006). One evident drawback of these experiments is that tumour dependency was induced artificially, through ectopic introduction of a hyperactive initiating oncogene in a normal tissue background. This is of course far from the evolutionary trajectory of spontaneous human tumours, in which the dominant oncogene does not necessarily correspond to the initiating driver alteration. However, the striking responses observed in these cellular and animal models anticipated those that would be observed, years later, in human patients treated with targeted therapies against individual oncoproteins.

The compelling concept proposed to account for these evidences is known as oncogene addiction. This definition

was first introduced by Bernard Weinstein in 2000, with particular reference to the observation that some cyclin D-overexpressing cancers reverse their malignant phenotype upon cyclin-D depletion by means of RNA interference (RNAi; Weinstein, 2000; Weinstein, 2002). In its original version, it postulated that cancer cells display either oncogene addiction (*i.e.* dependence on the prolonged activity of oncogenes) or gene hypersensitivity (*i.e.* sensitivity to the restored biochemical function of tumour suppressor genes); this was proposed to be due to the paradoxical, 'bizarre circuitry' typical of tumour cells, which in turn is the consequence of the altered mechanisms of cellular homeostasis fuelled by tumour formation and evolution. After more than 10 years, the oncogene addiction concept retains full validity and remains a paradigmatic example of how mechanistic studies can have immediate translational relevance.

Glossary

Cell-autonomous

A genetic trait in multicellular organisms in which only genotypically mutant cells exhibit the mutant phenotype. Conversely, a non-autonomous trait is one in which genotypically mutant cells cause other cells (regardless of their genotype) to exhibit a mutant phenotype.

Chimeric protein

A hybrid protein encoded by a nucleotide sequence that resulted from the fusion of two or more complete or partial genes.

Chronic phase, blast crisis (CML)

Chronic phase is the first phase of CML progression and presents with mild or even absent symptoms. Blast crisis is the last stage of CML, which behaves like an acute leukaemia and in which the number of blast cells is elevated in bone marrow and blood.

Deep sequencing

Technique to analyse nucleotide sequences with increased range, complexity, sensitivity and accuracy compared to standard, first-generation sequencing techniques.

Feedback loops

The causal pathways that lead from the initial generation of the feedback signal back to subsequently modify the event.

Fitness

High potential for survival under defined circumstances.

Genetic drift

Change in the frequency of a genetic variant, which takes place only by chance.

Genetic instability

A set of diverse genetic events, which cause either temporary or permanent changes within the genome.

High-throughput (Screening)

The process during which batches of compounds, expression libraries or RNA-interference libraries are tested for a defined activity on a certain target.

Open reading frame

A DNA sequence that contains a start codon but no stop codon and can potentially be translated into a protein.

Orthologue

Enzymes, which have the same activity.

Patient stratification

Definition of subsets of patients, which would benefit from a given therapy, with a shared biological characteristic identified with the use of biomarkers.

Transcriptional signature

Set of expressed mRNAs, which define the presence of a certain condition.

The Biological Bases of Oncogene Addiction

Essentially, three models have been put forward to elucidate the mechanisms of oncogene addiction at the molecular level. All of them take into account **cell-autonomous** (cancer-specific) properties and are known as (i) genetic streamlining, (ii) oncogenic shock and (iii) synthetic lethality (SL). Initially speculative, these theories were each experimentally validated and can explain different but complementary facets of this phenomenon.

Genetic streamlining

The genetic streamlining hypothesis stems from the well-established notion that cancer cells undergo constant **genetic drift** as a consequence of the selective pressure exerted by the tumorigenic process and by the tumour microenvironment. Because of this, cancer cells are thought to lose (or, better, actively dismiss) any cellular function that has proved to be non-essential for cell viability or does not provide any increase in

cellular **fitness** ('genome degeneration'). At the molecular level, this occurs presumably through a mutational burden of non-adaptive alterations or epigenetic modifications ('genetic load'). When the pressure exerted by the tumour microenvironment or by tumour-autonomous features remains constant, the genetic load in non-essential genes will have little effect on cell growth dynamics (Kamb, 2003). However, the widespread silencing of subsidiary functions renders cancer cells much more susceptible to acute perturbations: sudden changes in the composition of the surrounding stroma or inhibition of one or more of the pathways still active in cancer cells lead to rapid reduction in cellular fitness and collapse (Fig 1A). Theoretically, this process may produce an opposite outcome: an initially non-adaptive mutation can coexist as a passenger alteration along with driver mutations in the genome of a cancer cell until a new selective force – for example drug exposure – unleashes its potential to increase biological fitness in that particular circumstance; this, in some instances, can foster the emergence of resistant clones (see below).

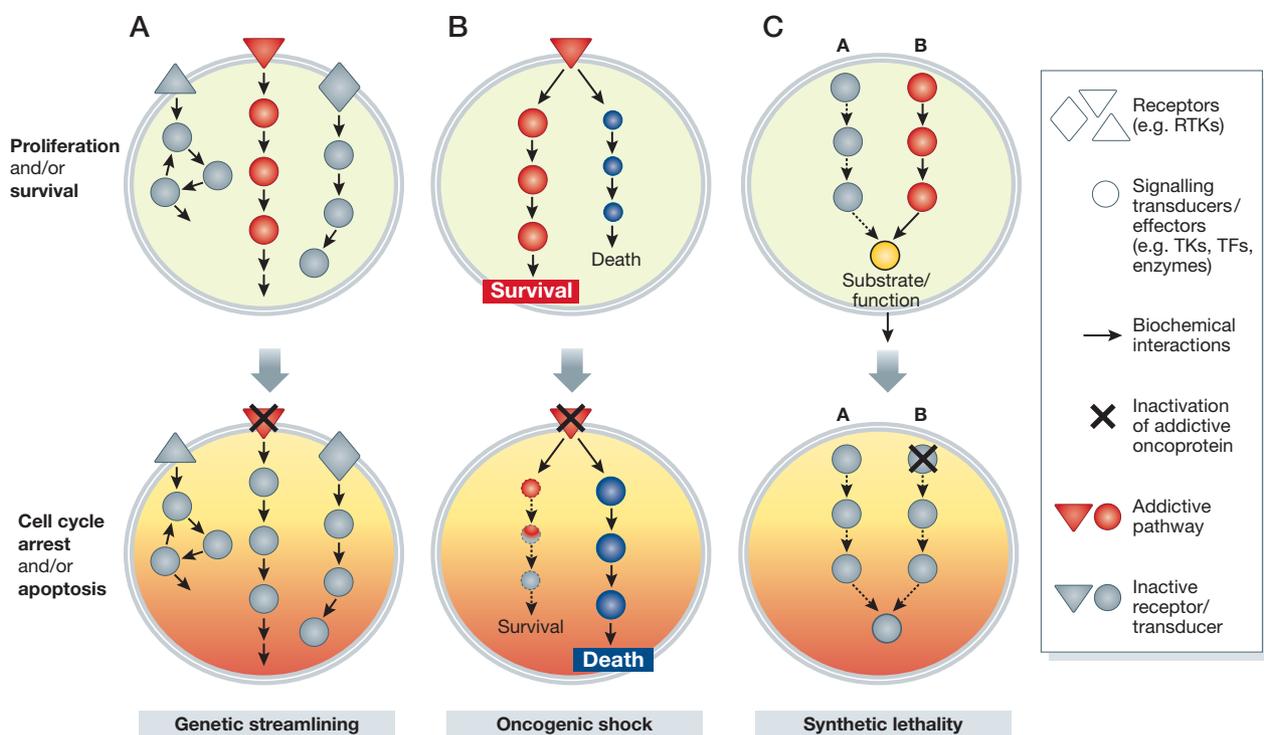


Figure 1. Models of oncogene addiction.

- The 'genetic streamlining' theory postulates that non-essential pathways (top, light grey) are inactivated during tumour evolution, so that dominant, additive pathways (red) are not surrogated by compensatory signals. Upon abrogation of dominant signals, there is a collapse in cellular fitness and cells experience cell-cycle arrest or apoptosis (bottom, red to yellow shading).
- In the 'oncogenic shock' model, additive oncoproteins (e.g. RTKs, red triangle) trigger at the same time pro-survival and pro-apoptotic signals (top, red and blue pathway, respectively). Under normal conditions, the pro-survival outputs dominate over the pro-apoptotic ones (top), but following blockade of the additive receptor, the rapid decline in the activity of survival pathways (dashed lines, bottom) subverts this balance in favour of death-inducing signals, which tend to last longer and eventually lead to apoptotic death.
- Two genes are considered to be in a synthetic lethal relationship when loss of one or the other is still compatible with survival but loss of both is fatal. In the top panel, biochemical inactivation of pathway A (grey) has no effect on cell viability because pathway B (red), which converges at some point on a common substrate or effector (yellow), has compensating activity. When the integrity of pathway B is disrupted (bottom), the common downstream biochemical function is lost and again cancer cells may experience cell cycle arrest or apoptosis.

Inactivation of signalling pathways in cancer cells after genetic drift may also occur at the biochemical or transcriptional levels as a consequence of chronic oncogenic signalling. Indeed, the unrelenting activity of dominant oncogenes is likely to be counteracted by a certain extent of reactive adaptation, including activation of compensatory pathways and positive or negative **feedback loops**. Using phosphoproteomic and gene expression profiling, we have recently demonstrated the presence of ‘sensitive’ and ‘indifferent’ pathways in cell lines addicted to the *MET* oncogene – encoding the Met tyrosine kinase receptor for hepatocyte growth factor (HGF) – or to epidermal growth factor receptor (EGFR). Met or EGFR inhibition in these settings results in the selective decline of RAS- and PI3K-dependent cascades, whilst many other signals known to affect Met- and EGFR-driven proliferation in non-addicted cells – including JNK, p38, STATs and NF- κ B – remain active or exhibit scant responses (Bertotti et al, 2009). In the context of genetic streamlining, this piece of information corroborates the notion that cancer cells host large arrays of indolent and functionally neutral pathways and small ensembles of functionally active, self-sufficient transducers. The presence of only a limited subset of operational signalling nodes and the absence of buffering circuits reveal the vulnerability of the oncogene addiction state.

Oncogenic shock

Settleman and colleagues have proposed a model referred to as ‘oncogenic shock’. The fundamental premise to this concept is that most dominant oncogenes are able to sustain at the same time both pro-survival and pro-apoptotic signals (Sharma and Settleman, 2007). This duality is an in-built property of normal cells, in which strong oncogenic insults can counteract excessive pro-mitogenic signals induced by the same molecule through concomitant induction of apoptosis. In transformed cells, this intrinsic apoptotic defense is disabled for many reasons, including the fact that the pro-survival signals that emanate from hyperactive oncoproteins tend to dominate over the parallel pro-apoptotic outputs (Fig 1B). A variety of experimental data, collected in diverse cellular and *in vivo* transgenic models, is in favour of this notion: for example the *MYC* oncogene displays apoptosis-inducing properties in low-serum conditions (Evan et al, 1992), which can be inhibited by overexpression of the anti-apoptotic BCL2 protein (Bissonnette et al, 1992) or PI3K/AKT pathway activation (Kauffmann-Zeh et al, 1997). In normal physiology, the pro-apoptotic function of *MYC* is apparent during development, as it causes the negative selection of T lymphocytes upon antigen stimulation (Shi et al, 1992). Similarly, continued overexpression of the *RAS* or *RAF* oncogenes in primary human cells induces cell cycle arrest through activation of the MAPK family members p38 and JNK, which in turn induce transcriptional upregulation of p53 or the cyclin-dependent kinase (CDK) inhibitors p16 and/or p21 (Fanton et al, 2001; Zhu et al, 1998). As an alternative to active induction of apoptosis, some cells react to relentless oncogenic signalling by entering a state of senescence, that is an irreversible condition of post-mitotic dormancy. *In vitro* and *in vivo* models have shown that hyperproliferating cells accumu-

late genomic lesions such as DNA double strand breaks (DSB), a process known as ‘replication stress’. In turn, DSBs engage the DNA damage response machinery, which activates the proto-typical effector ataxia telangiectasia mutated (ATM); this converges on either p53 or p21, triggering the proliferation blockade that typifies the senescent phenotype (Bartkova et al, 2006; Di Micco et al, 2006; Halazonetis et al, 2008).

The oncogenic shock hypothesis relies on the experimental observation that targeted disruption of signal-generating oncoproteins results in differential kinetics of downstream signal decay: anti-apoptotic effectors (such as ERKs, AKT and STATs) display rapid diminution of activity; conversely, death-inducing molecules (namely p38) display delayed accumulation. This temporal imbalance has been demonstrated in a variety of cellular systems driven by oncogenically active tyrosine kinases, including BCR-ABL, SRC and EGFR (Sharma et al, 2006; Sharma and Settleman, 2010b). The oncogenic shock hypothesis deserves at least two comments. First, it postulates that the apoptotic response observed following abrogation of addictive oncoproteins is an active process of signal-mediated induction of cell death; this is in contrast to the passive occurrence of signal deprivation predicted in the genetic streamlining model. Second, the ‘potency’ of the oncogenic signal in generating pro-survival and pro-apoptotic outputs seems to be more crucial than the temporal appearance of the dominant genetic lesion. While it can be intuitive to think that an initiating oncogene will be more influential as a dominant alteration than genetic lesions occurring subsequently during tumour evolution, we can also reasonably argue that addictive oncogenes with powerful pro-apoptotic activity are likely to arise late during the tumour’s natural history, when at least some apoptotic safeguards have been disengaged; otherwise, cells would die, and oncogene hyperactivity would be negatively selected.

Synthetic lethality

The theory of SL states that a gene A is in a synthetic lethal relationship with a gene B when loss of function of either gene A or gene B is fully compatible with cell viability, whereas, loss of activity of both A and B gene products is lethal for the cell (Kaelin, 2005). This notion is rather intuitive when genes A and B belong to alternative metabolic (enzymatic) chains with a common end-product; but, at least in principle, it can also be applied to signalling axes driving more sophisticated and integrated cellular functions, such as survival and proliferation (Fig 1C). The concept of SL dates back to the beginning of the last century and was initially applied to account for experimental data obtained in single-celled organisms, such as bacteria and yeast. In the past decade, it has been exploited for the characterization of **orthologue** enzymes involved in certain metabolic pathways in multi-cellular model organisms (*Caenorhabditis elegans*, *Drosophila melanogaster*) and, more recently, it has been extended to human tumour cell lines (Brough et al, 2011; Nijman, 2011). In cancer, SL is proposed to occur when alteration of a gene (*e.g.* its genetic silencing or pharmacologic inactivation) results in cell death only in the presence of another non-lethal genetic alteration (*e.g.* a cancer-associated mutation). Because the gene, that is synthetic lethal

in combination with the cancerous mutation is usually in its wild-type form, SL is also defined as ‘non-oncogene addiction’.

The SL working hypothesis led to the discovery of the synthetic lethal interaction between the *BRCA1* and/or *BRCA2* gene products and [poly(ADP-ribose)-polymerase] PARP-1 protein (Lord and Ashworth, 2008). *BRCA1* and *BRCA2* have a key role in homologous recombination (HR; Fig 2A), an

important pathway for DNA damage repair, and their inactivation results in defective restoration of DNA DSBs (see above). Single-strand breaks (SSBs) must also be repaired in *BRCA*-deficient cells, because SSBs can turn into lethal DSBs following DNA replication. The DNA repair pathway principally involved in repairing SSBs is base-excision repair (BER; Fig 2B), and one of the proteins essential for BER is PARP-1 (Rouleau et al, 2010).

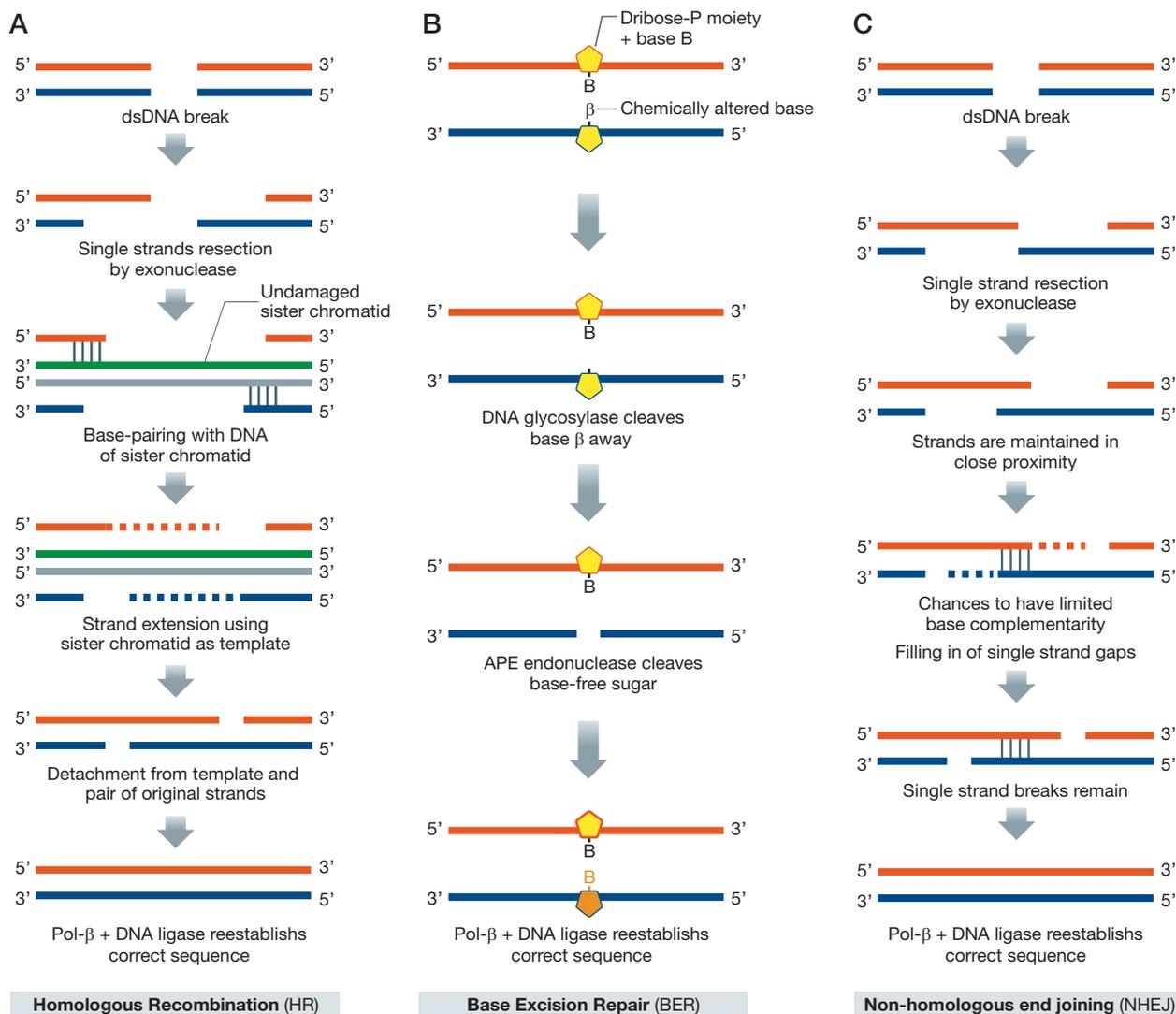


Figure 2. Modes of DNA damage repair.

- A.** HR safeguards genome integrity in late S/G2 phases of the cell cycle and relies on the ability to use the recently formed sister chromatids as a template to guide repair of the damaged strands. Exonuclease activity produces two single-stranded (ss) DNA ends, one at each side of a double-strand (ds) DNA break (top). Each of the two ssDNA ends pair with complementary sequences of the sister chromatid (unwinding of sister chromatids is not represented here for simplicity); elongation is then performed by DNA polymerase. Subsequent release from the sister chromatid, pairing of the elongated ssDNA ends, further elongation and, ultimately, ligation give rise to the wild-type sequence. *BRCA1* and *BRCA2*, among many other proteins, are part of the HR machinery.
- B.** BER senses chemically altered bases (β) with a minimal effect on double helix topology. Glycosylases first cleave the bond linking the base with the deoxyribose (middle top); this is in turn excised by an apurinic/aprimidinic endonuclease (APE) with high affinity for base-free sugars (middle bottom). DNA polymerase β replaces the missing nucleotide, which is finally ligated to reconstitute the correct sequence.
- C.** When homologous sequences are not available as templates, the ssDNA ends generated by exonucleases can be joined together by a small number of base pairs. This event if followed by filling of the gaps in each strand and ligation of any remaining ssDNA breaks. Of note, the sequence repaired by this NHEJ repair system lacks some of the bases originally present in the undamaged DNA and is therefore intrinsically error-prone.

It has been demonstrated that, when BER is inactivated (*e.g.* as a consequence of PARP1 inhibition), BRCA-deficient cells are unable to repair the DSBs that evolve from SSBs, and this leads to deadly defects in the genome (Bryant et al, 2005; Farmer et al, 2005). Interestingly, acquired resistance to PARP inhibitors in cells with BRCA2 loss-of-function alterations may result from late 'reverting' mutations, that is new deletions in the context of the BRCA2 gene that restore the normal **open reading frame** abrogated by the original genetic lesion, thus rendering the cells once again capable to perform HR (Edwards et al, 2008; Sakai et al, 2008). Of note, two studies recently suggested an alternative route for restoration of HR competence in BRCA1-deleted cells (Bouwman et al, 2010; Bunting et al, 2010). Tumour protein p53 binding protein 1 (TP53BP1, also known as 53BP1) is a component of the non-homologous end joining (NHEJ) repair system (Fig 2C), which acts by binding to chromatid breaks and triggers the formation of aberrant fusions between heterologous chromosomes. These fusion products can represent 'toxic' rearrangements and negatively affect cell viability. Mechanistically, 53BP1 blunts the ATM-mediated resection of the broken strands, ultimately impeding HR (Bunting et al, 2010). HR is the preferred repair type for DSBs in wild-type cells, whereas, in BRCA1-deficient cells (in which normal HR is compromised) the balance switches towards the NHEJ system. Suppression of residual HR function, for example through PARP inhibition, further enhances the 'toxic' switch to NHEJ, suggesting that neutralization of NHEJ might alleviate generalized DNA damage and counteract the effects of PARP inhibition. Indeed, RNAi-mediated depletion of 53BP1 reduces hypersensitivity of BRCA1 mutant cells to PARP inhibitors and leads to partial restoration of HR: in genetic terminology, 53BP1 loss is said to be 'synthetic viable' with BRCA1 deficiency (Aly and Ganesan, 2011; Kaelin, 2005). The clinical significance of these findings is still unclear: BRCA1-associated cancers often exhibit reduced expression of 53BP1, which might have predictive value as a potential biomarker of therapeutic resistance to PARP inhibitors (Bouwman et al, 2010).

In addition to applying the SL concept to tumour suppressor genes, an analogous strategy has been successfully implemented for dominantly acting oncogenes and specifically for KRAS. Considering that KRAS is a widespread target in human malignancies that remains substantially undruggable, the inhibition of its putative synthetic lethal partners was considered an affordable way to indirectly abrogate KRAS pro-tumorigenic activity. Using diverse screening approaches, several groups identified many putative SL interactors of KRAS. Singh et al used RNAi to deplete the KRAS protein in lung and pancreatic cancer cell lines harbouring mutated KRAS alleles; this identified KRAS-dependent cells (which reacted to KRAS silencing with growth impairment) and KRAS-independent cells (whose proliferative activity was not affected by KRAS silencing, despite the presence of activating KRAS mutations). The KRAS-dependent cells were characterized by the expression of proteins – including the tyrosine kinases SYK, RON, and integrin $\beta 6$ – that resulted in a differentiated epithelial phenotype and that, when inactivated, induced epithelial-mesenchymal transition and apoptosis (Singh et al, 2009).

A **high-throughput**, RNAi-mediated SL screen in KRAS-mutated cancer cells of different tissues revealed that the serine/threonine kinase STK33 is selectively required in these cells to sustain viability. This is mechanistically supported by the observation that STK33 suppresses mitochondrial apoptosis via S6K1-mediated inactivation of the pro-apoptotic effector BAD, although the signalling intermediates between STK33 and S6K1 are still to be characterized (Scholl et al, 2009). With a similar approach, Barbie et al discovered the anti-apoptotic role of the non-canonical I κ B kinase TBK1, an upstream activator of NF- κ B signalling, in KRAS-addicted cancer cell lines; this introduced NF- κ B-related signals as a 'co-dependent' survival pathway in KRAS-mutated cancers (Barbie et al, 2009). An independent genome-wide RNAi screen in isogenic cell lines differing only in the presence or absence of a KRAS mutation identified 77 SL candidate genes. Computational analysis supported an increased dependency of mutated cells on proteins involved in the mitotic machinery (*e.g.* polo-like kinase 1, PLK1) and the proteasome suggesting that KRAS mutation is associated with mitotic stress; intriguingly, gene expression analysis revealed a correlation between decreased expression of key mitotic proteins and longer survival of patients bearing tumours featuring a RAS **transcriptional signature** (Luo et al, 2009). Finally, the effects of depletion of different CDKs were studied in cells derived from mice engineered to endogenously express a mutant KRAS allele and in cell lines derived from KRAS mutated, non-small cell lung carcinomas (NSCLCs). Selective loss of CDK4 had the most prominent negative effect on the cancerous phenotype by inducing a strong senescence response (Puyol et al, 2010).

The ultimate validation of all these SL strategies will be evidence that patients with KRAS-mutated tumours clinically benefit from treatment with inhibitors of the identified SL partners. Selective CDK4 inhibitors are available for use in humans but so far have shown only modest therapeutic efficacy in unselected populations of patients with leukaemia or breast tumours. Based on the novel results discussed here, such inhibitors warrant future investigation in the context of KRAS-mutated NSCLCs (Krystof and Uldrijan, 2010; Shapiro, 2006). Similarly, PLK1 inhibitors are in the early phases of clinical development and, likely, will soon be tested in patients with KRAS-mutated cancers (Strebhardt, 2010).

Lastly, in addition to models that rely on cell-autonomous properties, recent data from Felsher and colleagues suggest that non-cell autonomous effects might deserve consideration in the context of oncogene addiction and 'synthetic lethal' heterologous cell-cell interactions. In particular, functional CD4⁺ T-lymphocytes appear to be needed for efficient induction of cellular senescence and/or inhibition of angiogenesis upon inactivation of the MYC or BCR-ABL oncogenes in mouse models of T cell acute lymphoblastic lymphoma and pro-B cell leukaemia, respectively (Rakhra et al, 2010).

The Clinical Correlates of Oncogene Addiction

Moving from reductionist approaches in oncogene-addicted cell lines to cancer therapy in potentially oncogene-addicted

Table 2. Targeted therapies in oncogene-addicted cancers

Name	Target	Disease	FDA first approval
Imatinib mesylate (Gleevec [®])	BCR-ABL, KIT ^{mut} , PDGFR ^{mut}	CML, GIST	2001 2002
Trastuzumab (Herceptin [®])	HER2 ^{ampl}	Breast cancer	1998
Erlotinib (Tarceva [®])	EGFR ^{mut}	NSCLC	2004
Gefitinib (Iressa [®])	EGFR ^{mut}	NSCLC	2003
Vemurafenib (PLX4032)	BRAF ^{mut}	Melanoma	Phase 3
Crizotinib (PF002341066)	ALK ^{transl}	NSCLC	Phase 3
Iniparib (BSI201)	PARP-1	BRCA-associated (hereditary) or 'BRCAness'-associated (sporadic) breast and ovarian cancer	Phase 2
Olaparib (AZD2281)	PARP-1	BRCA-associated (hereditary) or 'BRCAness'-associated (sporadic) breast and ovarian cancer	Phase 2/3

mut, mutant; ampl, amplified; transl, translocated; CML, chronic myeloid leukaemia; GIST, gastro-intestinal stromal tumour; NSCLC, non-small cell lung cancer.

tumours was a short step. In the past decade, the principle that some tumours depend on one single oncoprotein for their continuous growth – and the conclusion that this oncoprotein is ‘the’ target for therapeutic intervention – revealed itself as a master rule in translational cancer research and provided a rational framework to develop new targeted compounds for the treatment of various cancer types (de Bono and Ashworth, 2010; Stegmeier et al, 2010). This course of action is attested by a series of successful clinical stories that illustrate the impact of targeted therapeutics, when these are correctly used in selected cohorts of patients harbouring oncogene-addicted tumours. In the next paragraphs we will summarize the most relevant clinical examples, including well-proven paradigms now routinely applied to anti-cancer therapy and recent ‘from-bench-to-bedside’ achievements (Table 2).

BCR-ABL in chronic myeloid leukaemia

The small Philadelphia (Ph) chromosome was characterized for the first time in 1960 by Nowell and Hungerford as a peculiar cytogenetic aberration correlated with chronic myelogenous leukaemia (CML; Nowell & Hungerford, 1960). Little more than a decade later, it was apparent that Ph carried a balanced chromosomal translocation between the long arm of chromosome 9 (9q34) and the long arm of chromosome 22 (22q11), which produces a fusion transcript between the breakpoint cluster region-(BCR) and the gene coding for the Abelson (ABL) tyrosine kinase (de Klein et al, 1982; Rowley, 1973). Numerous reports followed providing convincing evidence that this constitutively active **chimeric protein** has a driving role in the pathogenesis and maintenance of CML; indeed, BCR-ABL was

the first rock-solid example of an addictive oncoprotein in human cancer at a time when the concept of oncogene addiction was still to come (Sawyers, 2009). Not surprisingly, much effort was then devoted to the search for chemical compounds able to inhibit BCR-ABL. The small-molecule tyrosine kinase inhibitor STI571 (imatinib; Gleevec[®], Novartis) showed promising results in pre-clinical models (Druker et al, 1996) and in 1998, the first Phase 1 study in **chronic phase**, Ph chromosome-positive, CML patients started at three university institutions in the United States. In almost 100% of patients, complete haematologic responses were observed and these results were confirmed in subsequent clinical studies. Even when clinical trials were conducted on patients experiencing **blast crisis**, the magnitude and frequency of clinical responses remained remarkably high, indicating that BCR-ABL maintained its causative function in sustaining malignant proliferation in all the phases of the disease. Together, these more than promising results led to the fast-track FDA approval of the drug in 2001 (Druker et al, 2001a, b).

Imatinib also selectively inhibits the receptor protein kinases KIT and PDGFR. Again, an approach based on a strong rationale, and some luck, led to the identification of activating mutations of the *KIT* gene in approximately 90% of gastrointestinal stromal tumours (GISTs); among KIT wild-type cases, 35% display activating mutations in *PDGFRA*. These observations supported the use of imatinib in advanced solid tumours, for which the functional significance of driver mutations in the late phases of disease was still a perplexing issue. As expected, FDA timely approved the use of imatinib in GISTs in 2002 (Antonescu, 2011).

The development of imatinib was the chance for a paradigm shift in the way cancer treatment was devised: medical oncologists confronted with the notion that cancer is a disease of genes not only at the molecular level, but also in terms of therapeutic strategies. It is worth noting that the deliberation of trialists to selectively recruit Ph chromosome-positive patients was unprecedented and set the stage for genetically based **patient stratification**, which today constitutes a cardinal principle in targeted trial design. Moreover, imatinib development was one of the first successes of medicinal chemistry screening programs, which led to the in-depth characterization of a small-molecule inhibitory compound against an oncogenically active tyrosine kinase (Lydon, 2009). Contrary to many traditional chemotherapeutic drugs, the discovery of imatinib was not a serendipitous occurrence but the long-sought result of a productive collaboration between academia and industry.

Amplification of HER2 in breast cancer

HER2 (also known as ErbB2) is the ligandless member of the EGFR family of receptor tyrosine kinases (RTKs). As much as 25–30% of breast cancers exhibit overexpression of the HER2 protein due to gene amplification, a feature that correlates with adverse prognosis (Valabrega et al, 2007). Moreover, gain- and loss-of-function approaches pointed to HER2 amplification as a driving event in the onset and progression of mammary tumours (Choudhury and Kiessling, 2004). The fact that a genetic alteration with a prognostic significance also plays a causative

role in sustaining the mammary malignant phenotype made HER2 an attractive target for therapeutic inhibition.

The first clinically relevant agent to block HER2 activity was a humanized monoclonal antibody against the HER2 extracellular domain (trastuzumab, Herceptin[®], Genentech). Despite accurate patient stratification based on assessment of HER2 protein overexpression and/or gene amplification, only about 30% of HER2-overexpressing metastatic breast cancer patients respond to trastuzumab monotherapy (Valabrega et al, 2007). Thus, HER2 amplification in breast cancer and BCR-ABL translocation in CML share a common basis (the occurrence of a genetic lesion that predicts response to its therapeutic inhibition) but they also have important dissimilarities. CML is a 'homogeneous' disease with a very high prevalence of the BCR-ABL translocation: almost all patients display the genetic characteristic that predicts response to imatinib, and almost all patients treated with imatinib do in fact respond to treatment. Conversely, breast cancer is a heterogeneous disease in which HER2 amplification defines only a subset of tumours, and among HER2-amplified tumours, responses are confined to only a fraction of cases. Together, these observations suggest that the 'addictive' potency of HER2 amplification in breast cancer is weaker than that of BCR-ABL in CML.

HER2-amplified tumours that do not respond to trastuzumab feature a condition of so-called 'primary' or '*de novo*' resistance, which is commonly due to the concomitant activation of alternative pathways that dominate over HER2 hyperactivation or blunt HER2-dependent signals. Examples include parallel activation of IGF1 receptor signalling, overexpression of EGF family ligands and hyperactivation of PI3K-based transduction cascades (Freudenberg et al, 2009). Consistently, a functional RNAi screen identified downregulation of PTEN as a mechanism of trastuzumab resistance. Activation of the PI3K pathway is caused by oncogenic mutations in the *PIK3CA* gene, encoding the catalytic subunit of PI3K, or loss of function of *PTEN*, encoding the phosphatase that opposes PI3K activity. Either condition is associated with poor prognosis after trastuzumab therapy, and the combined analysis of *PIK3CA* and *PTEN* status identifies twice as many patients at increased risk for progression compared to *PTEN* stratification alone. Thus, multi-parametric analysis of PI3K pathway activation may provide a biomarker to identify patients unlikely to respond to trastuzumab-based therapy (Berns et al, 2007; Stemke-Hale et al, 2008).

Mutated EGFR in non-small cell lung cancer

The small-molecule compounds gefitinib (Iressa[®], AstraZeneca) and erlotinib (Tarceva[®], Roche) block the catalytic activity of EGFR. Due to preliminary circumstantial evidence describing EGFR overexpression in lung cancer, these inhibitors were initially tested in unselected populations of non-small cell lung cancer (NSCLC) patients. Results were disappointing: the extent of clinical benefit from monotherapy with either drug hovered near the threshold for statistical significance, with response rates of approximately 10%, and the expected endpoints were not reached. Yet, the minor subpopulation of responsive patients had striking objective radiographic

responses and shared a number of clinical/epidemiologic characteristics (adenocarcinoma histology, East Asian ethnicity, a history of never smoking cigarettes and female gender), suggesting a common molecular background (Sequist and Lynch, 2008).

Retrospective genetic characterization of NSCLCs in responders and non-responders led to the seminal discovery that specific EGFR kinase domain-activating mutations significantly correlated with objective response to receptor inhibition. Once more, this correlation highlighted the crucial importance of mutationally activated kinases as anti-cancer drug targets. It also identified a genetic marker for the subset of NSCLCs highly responsive to EGFR inhibitors, providing momentum for the rational design of clinical trials in selected, EGFR-mutated patient cohorts. At least nine prospective studies involving patients with advanced NSCLC and activating EGFR mutations have substantiated the benefit of EGFR inhibition in EGFR-mutant lung cancer, with 50–70% response rates and significant improvements in progression free-survival (PFS) and overall survival rates (Pao and Chmielecki, 2010). In 2009, gefitinib was approved by the FDA for use in the advanced disease in all line setting. The main lesson learnt from this example is that there are cases in which targeted inhibition of tyrosine kinases is effective in only a small subgroup of patients, and kinase mutations represent the necessary predictors for patient stratification. Moreover, the low frequency of genetically defined responsive patient subsets raised the issue that a reliable representation of genetic diversity requires the consideration of a far broader sampling of individuals within a specific cancer type than previously assumed. This poses obvious logistical challenges for proper design, execution and interpretation of clinical trials based on previously identified molecules.

Similar to HER2, primary resistance also occurs in EGFR-mutated cancers. This can be due to additional alterations in the EGFR coding sequence, such as small insertions or deletions in exon 20 and rare mutations co-existing with classical activating mutations. Another mechanism, whereby, tumours with drug-sensitive EGFR mutations may not respond to treatment with EGFR inhibitors is the presence of other genetic lesions that affect signalling downstream of EGFR, including activating mutations in the *PIK3CA* gene or loss of *PTEN* expression (Pao and Chmielecki, 2010).

Mutated BRAF in melanomas

The RAF family member B-RAF is a serine/threonine kinase that is activated by RAS and, in turn, activates the MEK-ERK pathway. The notion that the *BRAF* gene is mutated – with variable frequencies – in many human tumour types dates back to 2002 and, since then, a panel of somatic missense mutations resulting in different levels of constitutive biochemical activity has been described (Arkenau et al, 2011; Dhomen and Marais, 2007). Specifically, cutaneous melanomas display a 50–60% prevalence of the V600E (*BRAF*^{V600E}) mutated allele. The widespread incidence of this genetic alteration, the high extent of RAF catalytic hyperactivation produced by this amino acid-specific mutation and its strong transforming properties *in vitro*

and *in vivo* are powerful indicators of its driver function in melanomagenesis (Gray-Schopfer et al, 2007). In the past few years, several small-molecule inhibitory compounds against BRAF have been isolated. In particular, PLX4032 (Plexxikon), which exhibits high selectivity specifically for the mutationally activated BRAF^{V600E} allele, showed promising results in the pre-clinical setting. Accordingly, a Phase 1 trial with an extension cohort reported 81% overall response rate (complete or partial response) in mutated patients (Flaherty et al, 2010). Because of this early promise, PLX4032 entered a Phase 3 trial directly after Phase 1. The durability of response to PLX4032 is still under evaluation (BRIM3 trial; ClinicalTrials.gov number NCT01006980). Median PFS in the Phase 1 extension cohort has been estimated to be at least 7 months, which compares favourably with a PFS of less than 2 months in historical datasets of advanced melanoma patients. However, tumour regrowth has been documented to occur in many patients, which underscores the frequent emergence of resistance in this therapeutic setting (see below) and highlights the need for improved long-term efficacy as a crucial goal of ongoing Phase 3 trials.

Interestingly, PLX4032 induces a paradoxical activation of the RAF-MEK-ERK pathway upon inhibition of wild-type BRAF in a RAS-mutant context due to the formation of active BRAF-CRAF or CRAF-CRAF dimers. This calls for caution regarding the use of RAF inhibitors in RAS-mutant tumours, including a minority of melanomas that harbour RAS alterations (Poulikakos and Rosen, 2011).

EML4-ALK in non-small cell lung cancer

The most recent insight into the successful clinical application of the oncogene addiction principle is the use of a small molecule inhibitor of ALK (anaplastic lymphoma kinase) in NSCLC patients. In 2007, a small interstitial deletion and inversion within chromosome 2p [inv(2)(p21:p23)] was identified that results in the synthesis of a fusion protein between EML4 (echinoderm microtubule-associated protein like-4) and ALK in 2–7% of NSCLCs. Biochemical and functional experiments revealed that the fusion protein harbours a constitutively active ALK and exhibits tumourigenic potential (Sasaki et al, 2010). Despite the appreciably lower incidence of ALK mutations in NSCLCs when compared, for example to mutated BRAF alleles in melanomas, the strong oncogenic activity of EML4-ALK offered a solid pre-clinical rationale for therapeutic targeting (McDermott et al, 2008). The ALK inhibitor PF-02341066 (crizotinib, Pfizer) has produced excellent results in a Phase 1 trial in NSCLC patients, with a 57% response rate in 82 ALK-rearranged patients (Kwak et al, 2010). Rates of disease control (response or stable disease for at least 8 weeks) exceeded 90% with negligible toxicity. Similar to the case of BRAF inhibition in BRAF-mutant melanomas, the compelling nature of these findings prompted the execution of a Phase 3 trial without an intermediate Phase 2 study (Gerber and Minna, 2010).

The rapid implementation of Phase 1 information into large, randomized Phase 3 trials is a clear sign of the power of early studies when these are supported by a strong biological rationale and by highly significant responses. However, the

ethical aspects of such an accelerated procedure remain controversial: if patients with incurable disease, whose tumour have the addictive mutation, are informed of the efficacy of the experimental drug, they will want (and deserve) access to the new treatment and may not accept random assignment to a poorly effective and toxic conventional therapy. This challenge is one of the many thorny issues that oncologists have to face when dealing with potentially effective targeted therapies (de Bono and Ashworth, 2010).

PARP-1 in ovarian and ‘triple negative’ breast cancer

As discussed above, PARP-1 inhibition is synthetic lethal in combination with faulty HR mechanisms. A Phase 1 trial conducted in 2009 demonstrated the anti-tumour activity of the small-molecule PARP inhibitor olaparib (AstraZeneca) in a population of cancer patients (ovarian, breast and prostate) enriched for *BRCA1* or *BRCA2* mutations, with *BRCA* mutation carriers exhibiting radiographic response or meaningful disease stabilization (stable disease for a period of 4 months or more; Fong et al, 2009).

Triple-negative breast cancer (estrogen receptor-negative, progesterone receptor-negative and not overexpressing HER2) is an aggressive subtype of breast cancer that shares clinical and pathological characteristics with hereditary *BRCA1*-related mammary tumours. In sporadic triple-negative breast cancer, the function of *BRCA1* is defective due to promoter hypermethylation, overexpression of negative regulators or other defects in HR pathways. Together, these alterations define a context of ‘BRCAness’ that suggests SL with PARP inhibitors. Indeed, a Phase 2 study compared the efficacy of a classical platinum-containing doublet with or without the PARP inhibitor iniparib (Sanofi Aventis) in a cohort of patients with advanced triple-negative breast cancers, documenting an increase in overall response rate from 32 to 52% with the addition of iniparib to chemotherapy (O’Shaughnessy et al, 2011). However, interim results from a Phase 3 trial, which were made available to the public in early 2011, were disappointing: similar to the Phase 2 study, the drug was used in a randomized trial with patients receiving gemcitabine and carboplatin with or without iniparib. No improvement in overall survival or progression-free survival was found in patients receiving iniparib as a first-line therapy (ClinicalTrials.gov number: NCT00938652). Likely, the increase in the size of the study population that characterizes Phase 3 trials has somehow diluted the occurrence of the BRCAness phenotype in triple-negative breast cancers, and specific response biomarkers are needed for better patient stratification.

A Common Theme: The Emergence of Secondary Resistance

The emergence of secondary (acquired) resistance at some point during treatment soon became apparent when a clinical trial of imatinib in blast-crisis CML patients showed that some subjects had developed clinical insensitivity to the drug after remarkable but transient remission. Initial studies indicated that the average

chronic-phase patient using imatinib has a risk of approximately 10%/year of relapsing into blast crisis. Analysis of BCR-ABL sequences in the myeloid clones of patients with imatinib-resistant, relapsed disease indicated a high frequency of mutations in the BCR-ABL gene. The prototypic amino-acid substitution (T315I) produces a steric hindrance in the ATP-binding pocket of the kinase, which interferes with insertion of imatinib into the cavity ('gatekeeper mutation'). Other mutations lock the BCR-ABL kinase domain in an active position, preventing binding of imatinib (Shah and Sawyers, 2003). In this case, chemicals able to bind this conformation are expected to achieve full inhibitory potential. At least two molecules, dasatinib (Sprycel[®], Bristol-Myers Squibb) and nilotinib (Tasigna[®], Novartis), display this property and are now used for the treatment of relapsing, resistant CML patients. In a minority of cases, imatinib resistance results from amplification of the BCR-ABL gene, which leads to increased levels of the corresponding protein product (Engelman and Settleman, 2008).

The acquisition of secondary mutations that prevent drug binding to the kinase catalytic cleft has been documented as a cause of secondary resistance also for other targets and in other oncogene-addicted tumours, including mutant EGFR and EML4-ALK in NSCLCs as well as mutant c-KIT in GISTs (Engelman and Jänne, 2008; Stegmeier et al, 2010). Alternatively, other oncogenes can undergo genetic alterations to produce aberrant signalling in lieu of the pathways that are no longer sustained by the inhibited target. Some gefitinib-resistant NSCLCs, for example harbour amplifications of the *MET* oncogene, which triggers PI3K survival signals via trans-phosphorylation of ERBB3 (Engelman et al, 2007). As previously discussed, BRAF-mutant melanomas treated with RAF inhibitors tend to regrow and progress after excellent initial response. A number of recent papers have revealed potential mechanisms of acquired resistance to RAF inhibitors: again, these mechanisms entail genetic alterations that circumvent target blockade by acting either on the target itself (BRAF amplification) or on analogous pathways (NRAS amplification or overexpression of COT, a MAPK pathway agonist). Some of these resistance-inducing alterations have been also detected in tumour material from relapsed patients (reviewed in Poulikakos and Rosen, 2011).

The common theme of all these examples is that the inactivated target is bypassed by compensating lesions that may act either in a vertical or horizontal fashion: in the former model, secondary alterations within the same upstream target re-stimulate the downstream signalling flux along the same, previously inhibited pathway; in the latter, parallel axes are activated that substitute for the blocked signalling (Fig 3B and C). Thus, **genetic instability** may fuel the appearance of oncogenic lesions that are evolutionarily selected to drive tumour survival and growth upon the selective pressure of drug exposure. In this scenario, an intriguing hypothesis is that secondary resistance is in fact nothing but the clonal selection of a minor subpopulation of cells that pre-exist before treatment and harbour *a priori* resistant alleles: drug exposure eliminates the bulk of sensitive cells, eventually leading to expansion of the resistant clones. This assumption was experimentally validated in several tumour settings (Fig 3A). At least some imatinib-

resistant CML clones are thought to occur at low frequency prior to treatment and experience clonal selection upon imatinib exposure (Hofmann et al, 2003; Shah et al, 2002). Similarly, the EGFR T790M mutation can be detected at low levels in patients with EGFR-mutant NSCLCs (Maheswaran et al, 2008). Finally, rare cells exhibiting amplification of the *MET* oncogene are present in NSCLCs before drug exposure. Such cells are intrinsically resistant to EGFR inhibitors and emerge during the course of therapy through a positive selection process that is dramatically accelerated by the environmental availability of the Met ligand HGF (Turke et al, 2010).

Other mechanisms of drug resistance have been demonstrated at the pre-clinical level. The concept that cancer cell populations are inherently heterogeneous and that individual cells respond differently to drug treatment has been supported by the finding that human cancer cell lines contain a small subpopulation of reversibly 'drug-tolerant' cells, displaying reduced drug sensitivity only for a limited period of time. This transient condition requires IGF1-R signalling and an altered chromatin state, which indicates that this phenomenon is chiefly sustained by epigenetic, rather than genetic, mechanisms (Sharma et al, 2010a). Similar to antibiotic-tolerant bacterial strains, which also display a provisional ability to bear potentially lethal stresses, the reversible insensitivity of drug-tolerant cells to initial drug exposure likely allows the production of more stable resistance mechanisms. Whether this dynamic switch between reversibly tolerant and sensitive tumour subpopulations also occurs *in vivo* in drug-treated tumours remains to be established.

Outlook

Since its inception, the concept of oncogene addiction was widely accepted amongst molecular oncologists. Maybe for the first time in cancer research, biological evidence has been almost directly implemented into an operative vision for the rational treatment of cancer. Yet, experimental and clinical facts raise a number of issues that warrant further investigation. For example the biological outcomes produced by inhibition of oncogene-addicted cells (cell-cycle arrest, senescence, differentiation or apoptosis) appear to be context-dependent, and the signalling circuitries beneath these cell-specific responses still have to be explored. Understanding the mechanisms that regulate cytostatic *versus* cytotoxic responses will have important therapeutic implications. Although extensive, our knowledge of signalling networks is still rudimentary. We believe that worthwhile information will be provided by the ongoing development of new technologies for large-scale, unbiased analyses: genome-wide functional screens, **deep sequencing**, mass spectrometry and advanced imaging approaches will offer extensive ground for hypothesis generation and for additional target discovery. From a mechanistic perspective, we predict that the incoming technological armamentarium will encourage a switch from an oncogene-centered to a network-based interpretation of data; this, in turn, will likely provide full awareness of the complex cellular circuits that generate and maintain oncogene addiction.

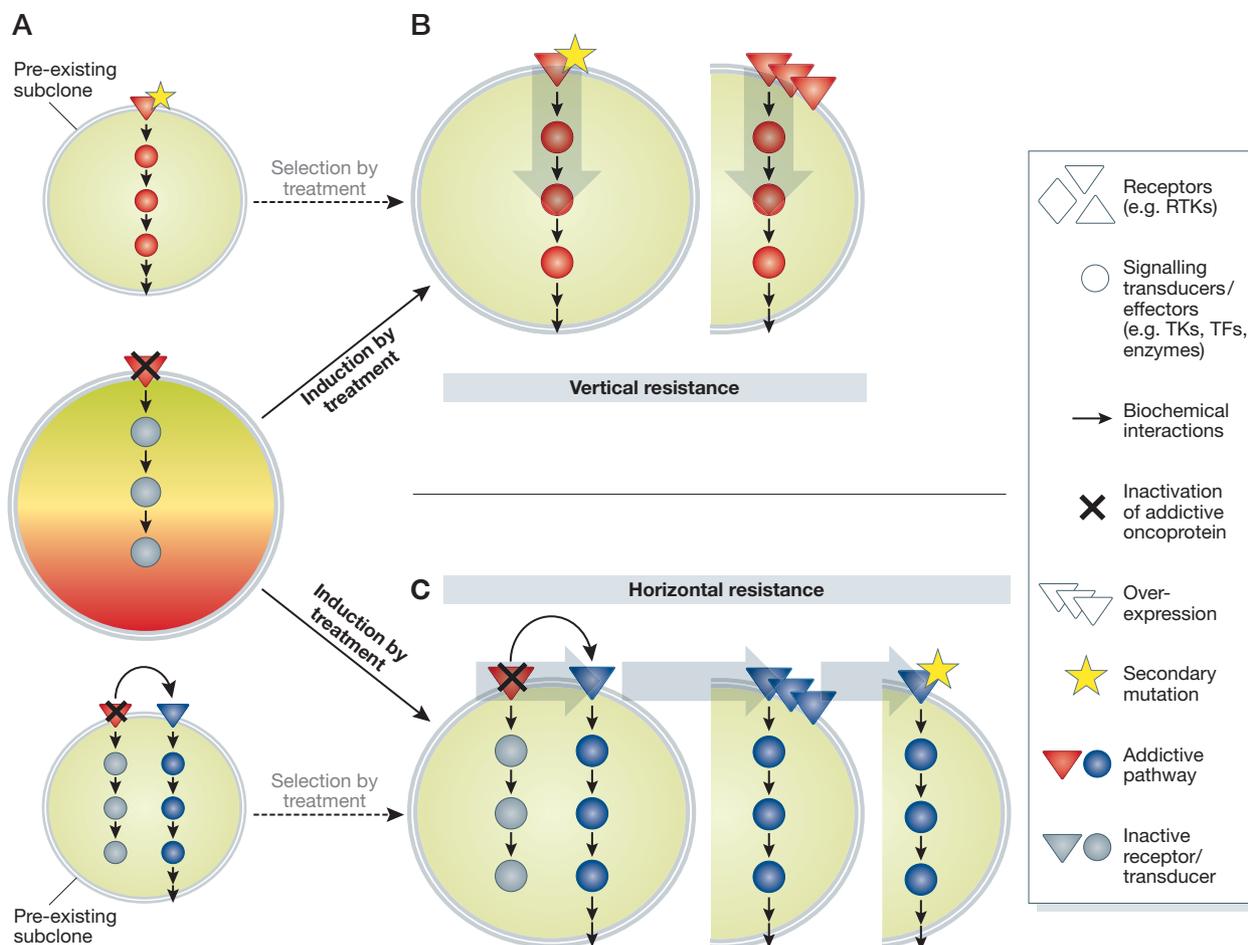


Figure 3. Models of secondary resistance.

- A.** When the additive oncoprotein is inhibited, viability is seriously compromised and cancer cells stop proliferating or die (middle). Addiction to a certain oncogene might not be a homogeneous characteristic of the cancer cell population, and drug-insensitive subclones (top and bottom) may co-exist with sensitive cells.
- B, C.** Fuelled by genomic instability, targeted treatment of addicted cells may result in induction (arrows, solid line) of secondary resistance through either mutation (star) or amplification/overexpression of relevant signalling nodes. This may happen in a vertical fashion, by alterations involving downstream effectors of the original additive pathway (B), or in a horizontal fashion, when a parallel signalling axis surrogates target blockade (C). If resistant clones are already present at the beginning of treatment (panel A), these may be selected by drug exposure (arrows, dashed line) until they outcompete sensitive cells, resulting in 'acquired' resistance as well.

At the clinical level, much remains to be done to optimize molecular information for patient stratification and treatment during trial execution. Experimental drugs are typically assessed in patients with late-stage disease and often for compassionate treatment, when all prior therapies with standard agents have failed. While ethically impeccable, this approach inevitably underestimates the effects that the same investigational drug could exert in less advanced cancers. Detection of predictive biomarkers requires genetic or expression analysis on tumour material; similarly, assessment of pharmacodynamic biomarkers (which measure the near-term treatment effects of a drug on its target) may imply acquisition of bioptic samples. Because these assays are preferably carried out concomitantly with therapy, re-biopsy approaches should

be encouraged. However, the clinical trial community is reluctant to design studies that require additional tissue samples due to reasonable concerns about more difficult patient enrolment. We appreciate that, when moving from the preclinical to the clinical setting, the ethical and logistical challenges become enormous; but we also believe that the psychological and physical burden that patients will bear for standardized provision of human material will be rewarded by application of better personalized therapies. 'Flexible' and 'adaptive' trials, in which molecular information and clinical data are handled quickly and effectively for real-time assignment of each individual to the most suitable therapeutic regimen, will be key to increasing life expectancy in cancer patients.

Pending issues

Mechanistic/computational studies aimed at better defining the network architecture of the pathways that sustain oncogene addiction.

Detailed cataloging and characterization of the mechanisms underlying primary and secondary resistance in cells, animal models and humans.

'Adaptive' trial format design, favouring fast and easy validation of clinical observations in the laboratory as well as streamlining of experimental information from bench-to bedside.

Development of new reliable biomarkers of response to targeted agents to optimize real-time patient stratification.

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