



## Review

## From molecular changes to customised therapy

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**Abstract**

The revolution in molecular methods has allowed the development of approaches whereby cancer-specific changes can be utilised for targeted therapies. Gene therapy strategies include mutation compensation for correction of cancer-associated defects, and molecular chemotherapy for delivering toxic substances locally to tumour cells. Viruses which replicate only in tumour cells represent a powerful novel approach undergoing intensive development, with some exciting clinical results. Cancer vaccines are promising, although the final clinical evidence is still pending. Monoclonal antibodies, in conjunction with chemotherapeutics, are becoming standard therapy. Recently, the first small molecular inhibitors have made clinical breakthroughs. Importantly, the large amount of information available on genes differentially expressed in cancer cells, allows correlation of prognosis and treatment responses to molecular changes. Thus, the future of cancer treatment could be customised treatment based on the molecular properties of the tumour, utilising combinations of novel and conventional agents. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** Tumour suppressor genes; Peutz–Jeghers syndrome; Microsatellite instability; Gene therapy; Adenovirus; Enzyme inhibitors; Cancer vaccines; Immunotherapy; Monoclonal antibodies; Cancer

**1. Introduction: cancer as a molecular disease**

The identification of mutations in tumour suppressor and oncogenes as the cause of cancer immediately suggested rational means for treating the disease. If such changes could be taken advantage of to limit the anti-tumour effect to cancer cells, damage to normal tissues could be minimised. The improvements seen in recent decades in molecular and genetic methods have given powerful tools to researchers. Consequently, we now have large amounts of data on cancer-associated genes, their expression profiles and mutations seen in cancer cells. The next challenge will be to try to convert the knowledge into therapeutic benefit for patients (Fig. 1).

An important realisation is that metastatic cancer arises as a sequence of epigenetic and genetic changes, each of which increases the aggressiveness, viability or ability of the clone to escape immune defences. The ‘gatekeeper’ hypothesis suggests that each cell type has a guardian, the mutation of which is required for the initiation of carcinogenesis [1]. If confirmed, this phe-

nomenon could be useful for intervention. Furthermore, taking advantage of themes common to various cancer types, such as *TP53* or *Rb/p16* pathway mutations or cyclo-oxygenase 2 overexpression, could result in widely applicable antitumour strategies. Many genes important in tumour development have been identified through research on hereditary cancer syndromes. One such example is *LKB1*, hereditary mutations of which cause Peutz–Jeghers syndrome [2,3], and somatic mutations are seen in sporadic cancers [4–7].

Molecular diagnosis is not a novel concept. In fact, karyotyping has been used for decades for the classification of leukaemias. Importantly, we now have effective tools such as array and sequencing-based methodologies which allow detailed molecular analysis of each tumour. However, there is much work to be done in correlating molecular profiles with treatment responses and prognosis.

12% of colorectal cancers display a phenomenon termed microsatellite instability or MSI [8,9]. The underlying cause of MSI is the biallelic inactivation of mismatch repair genes [10], which are normally responsible for correcting mistakes that arise during DNA replication. 5-fluorouracil (5-FU) acts by interfering with nucleic acid synthesis and thus its effect could be different on MSI+ tumours. Therefore, we followed

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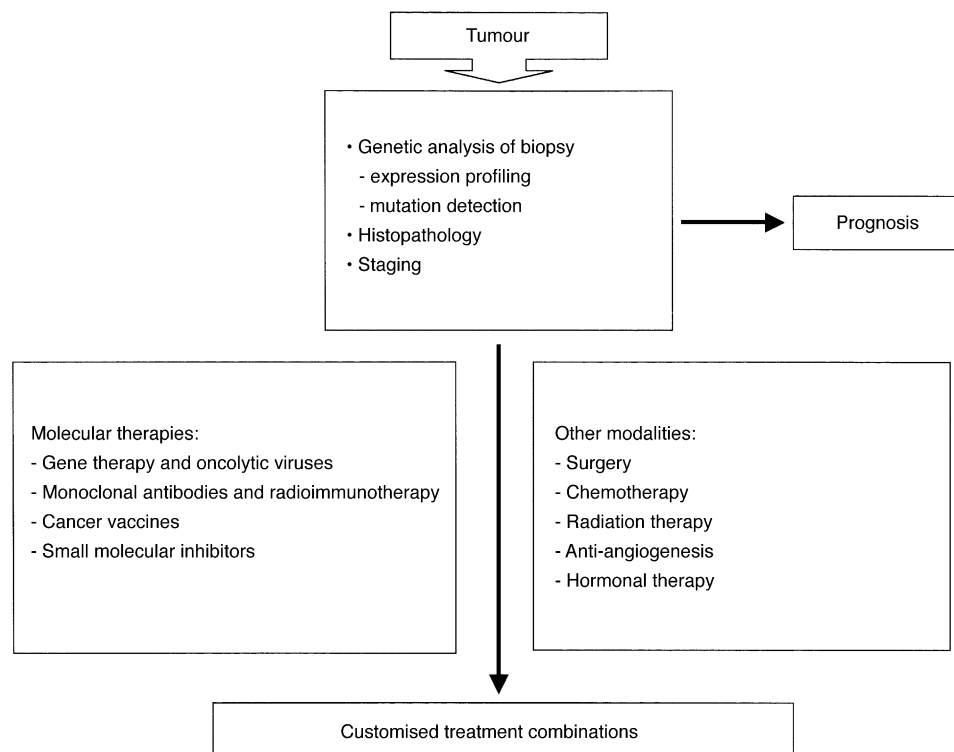


Fig. 1. From molecular changes to customised therapy. A schematic presentation of how molecular-based cancer treatment could be realised. Detection of mutations and epigenetic changes in tumour suppressor and oncogenes could be used for determining the best treatment combination for each patient individually. In addition, molecular correlates could improve the estimation of prognosis.

colorectal cancer patients, who had received adjuvant 5-FU [11]. The 3-year recurrence-free survival of MSI+ patients was 90%, while it was 43% for MSI– patients. Furthermore, we performed a preliminary investigation of Stage IV MSI+ patients and the results suggested a good response to 5-FU. These findings suggest that 5-FU is safe and could be very useful for the treatment of MSI+ colorectal cancer patients [12]. Thus, molecular correlates like MSI, the analysis of which is not labour-intensive or expensive, could have a significant effect on treatment response and prognosis.

Chemotherapy and radiation therapy are widely used for treatment of metastatic cases of cancer, but their tumour selectivity is relatively low. In contrast, molecular-based therapeutics are designed to be specific for tumour-associated features. Available approaches include gene therapy, monoclonal antibodies, cancer vaccines and small molecular inhibitors (Table 1).

## 2. Cancer as a target for gene therapy

Initially, gene therapy was developed for the treatment of hereditary and metabolic disorders, but many of the early approaches were characterised by excessive optimism. Gene transfer or therapeutic effects were not demonstrated. As summarised in the landmark Orkin–Motulsky report in 1995, basic research into vector

biology was necessary to improve target cell transduction, which remains the main obstacle in the field to date. Meanwhile, the focus of gene therapy shifted from hereditary to more common acquired disorders, and nearly 80% of patients treated in trials now are cancer patients. In addition, with cancer the aim is to kill the target cells, which is much easier than long-term expression of proteins at therapeutic levels.

The main approaches that have been used in cancer gene therapy include mutation compensation, molecular chemotherapy, and replication-competent viruses. The most frequent target for *mutation compensation* has been *TP53*, one of the most commonly altered genes in human cancers. A vector can be used to introduce the healthy *TP53* gene into cancer cells in order to induce cell cycle arrest and apoptosis. This approach has been widely tested in human trials, with excellent safety data, but the efficacy of this approach has been disappointing [13]. Concurrently, the field has advanced significantly and shortcomings of this strategy have become evident. With a non-replicating virus, it is extremely difficult to transduce every cell in a solid tumour, a requirement for efficacy with mutation compensation strategies.

Another widely used approach is *molecular chemotherapy* or gene-directed enzyme prodrug therapy. The classic example is Herpes Simplex virus type I thymidine kinase (TK), which catalyses the phosphorylation of systemically administrable non-toxic ganciclovir (GCV)

Table 1  
Molecular-based therapies undergoing clinical evaluation

Approach	Examples	Target	Features	Clinical success
<b>Gene therapy</b>				
Mutation compensation	Ad- <i>TP53</i>	p53-mutant cells	Reintroduction of p53 causes apoptosis	NSCLC
Molecular chemotherapy	Ad- <i>TK</i>	Infected cells	Direct and bystander anti-tumor effects by converted prodrug	Glioma
Replicative viruses	dl1520	p53/p14ARF-mutant cells	Replication kills cells and allows increased tumor penetration	SCCHN
Monoclonal antibodies	Trastuzumab	ErbB2	With chemotherapy	Breast cancer
	Cetuximab	EGFR	With radiation or chemotherapy	SCCHN, CRC, pancreatic cancer
	Rituximab	CD20	Alone or with chemotherapy	Lymphomas, leukaemias
Radioimmunotherapy	Tositumomab-I131	CD20	Antibody targets radiation to tumour	Non-Hodgkin's lymphoma
Cancer vaccines	Activation of immune cells	Dendritic cells	Activate with tumour antigen	Melanoma, breast, gastrointestinal cancer
	Modulation of tumour cells	Tumour cells	Modified tumour cells elicit immune response	Melanoma, renal cancer, CRC
Small molecular inhibitors	STI571	BCR/ABL, c-kit, PDGF	Blocks ATP-binding site	CML, ALL, GIST
	ZD1839	EGFR, ErbB2	Blocks ATP-binding site	NSCLC

Ad, adenovirus; NSCLC, non-small cell lung cancer; TK, Herpes simplex virus type I thymidine kinase; SCCHN, squamous cell cancer of the head and neck; CRC, colorectal cancer; CML, chronic myeloid leukaemia; ALL, acute lymphatic leukaemia; GIST, gastrointestinal stromal tumour; EGFR, Epidermal Growth Factor Receptor; PDGF, Platelet-Derived Growth Factor; ATP, adenosine triphosphate.

into GCV-monophosphate, which undergoes further modifications by cellular enzymes into toxic GCV-triphosphate. Furthermore, GCV-triphosphate can escape producer cells via gap junctions and thereby confer a bystander effect, allowing cells not directly affected with the vector to be killed. A limitation of molecular chemotherapy is the promiscuity of the vector systems used. Transduction of normal tissues causes toxicity and thus targeting of the vector and/or expression of the gene is crucial. Fortunately, transcriptional and transductional targeting is becoming more sophisticated, and molecular chemotherapy continues to be an attractive option with clinical potential.

One of the most exciting current approaches is *replication-competent viruses* (Fig. 2). Their development has been based on the need for more effective tumour penetration. In theory, subsequent rounds of replication allows these agents to penetrate deeper into the tumour until all of the cells have been infected [14]. Each infected cell can produce thousands of virions and therefore there is a tremendous potential for amplification of the effect. Obviously, it is crucial to limit the replication of the agent to tumour cells.

It has been suggested that some viruses, such as Reovirus, Vesicular stomatitis and the Newcastle disease virus, could have intrinsic selectivity for replication in tumours [15–17]. The basis of the tumour selectivity of Reoviruses may be in part due to activation of the *Ras* pathway and defective interferon pathways could be involved with some of the other viruses, but the basic

biology of these viruses is largely unknown. The most promising examples of replication-competent viruses involve intensively studied viruses with strong oncolytic potential, such as the adenovirus.

### 3. Adenoviral cancer gene therapy

The adenovirus (Ad) is perhaps the most studied viral gene transfer vector, which facilitates the creation of

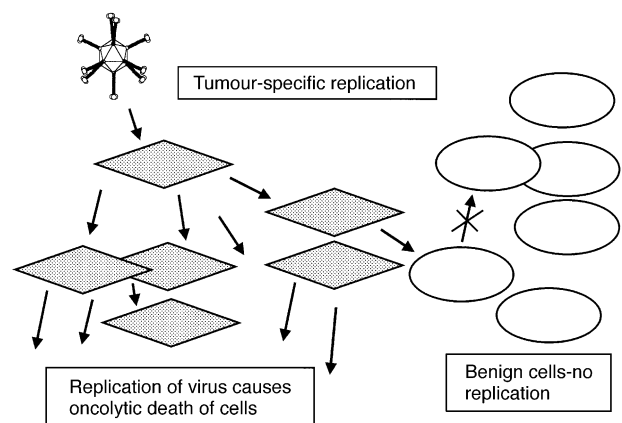


Fig. 2. Conditionally-replicating adenoviruses. Tumour cell-specific oncolytic cell killing is achieved by deletion of genes superfluous for replication in tumour cells (diamond shapes). Alternatively, tumour- or tissue-specific promoters can be used for controlling expression of crucial adenovirus genes. Subsequent rounds of replication allow effective penetration into the tumour.

recombinant agents. Advantages of Ad include unparalleled transduction efficacy of dividing and quiescent cells, natural tropism to a wide range of epithelial tissues and easy production of high titres [18]. The rate-limiting factor for infection is binding to the primary receptor, the coxsackie-adenovirus receptor (CAR) [13]. Ad DNA is transported to the nucleus, but does not integrate and thus the risk of mutagenesis is small, and while the short-term expression is undesirable for hereditary disease, it is unproblematic in oncology where the goal is to kill infected cells. Ad is very immunogenic which could be useful for inducing an immune response against the tumour.

Unfortunately, it is becoming increasingly clear that expression of CAR on primary human tumour cells, in various tumour types, is highly variable and often low [13]. Thus, it is crucial to analyse primary tumour samples in addition to cell lines [19,20]. Importantly, various approaches have been developed to circumvent the dependence on CAR. For example, bivalent retargeting molecules have been used to mediate interactions between Ad fibre and a tumour-associated receptor [21]. More sophisticated targeting can be achieved by genetically incorporating peptides into the Ad capsid [22], so that all newly produced viral particles display the modified tropism. In addition, advanced generation Ad vectors allow non-invasive imaging of gene expression and localisation [23]. This may help produce important correlative data from early phase trials, allowing estimation of potential efficacy before phase II and III trials.

Traditionally, Ads in oncology have been used as replication-deficient vectors for transporting genes into tumour cells. Safety data has been extremely good, but in most cases tumour transduction has been insufficient to achieve clinical responses [13]. However, in a glioma clinical trial, patients were randomised to receive Ad-*TK*, packaging cells for retrovirus with *TK* or Ad-*LacZ* (a marker gene) [24]. Injections were performed into the operation margins, followed by GCV treatment. The patients injected with Ad-*TK* survived significantly longer than the other groups, suggesting that clinical responses can be achieved even with a relatively basic approach. It seems likely there will be routine applications for non-replicating viruses, when local treatment can be applied and the tumour burden is small. In addition, the enhancement of infectivity and combination treatments could dramatically improve the results.

#### 4. Conditionally-replicating adenoviruses

The replication of *conditionally-replicating Ads* (*CRADs*) (Fig. 2) is controlled by specifically engineered deletions or tissue-specific promoters [25]. As Ad is an oncolytic virus, cells allowing replication die in the process. ‘Virotherapy’ with Ad was first explored in

cervical cancer patients in 1956, soon after the description of the agent [26]. Tumour responses were frequently seen, but the lack of cures led to few continuation studies.

This approach resurfaced in force when a CRAD called dl1520 was characterised as an anticancer agent [27]. dl1520 does not express E1B55kD protein, which participates in the binding and inactivation of p53 in cells infected with Ad. Thus, it was hypothesised that this virus could only replicate in cells in which *TP53* or its counterpart *p14ARF* was mutated. It remains controversial how precisely this holds true in practice. Furthermore, the lack of E1B55kD reduces the replicative ability of the agent, which may have contributed to the low single-agent efficacy seen in clinical trials [28]. In contrast, dl1520 has been very promising in combination with chemotherapeutics [29]. Objective responses (> 50% reduction in tumour size) were seen in 63% of patients with recurrent head and neck cancer, and 26% had complete responses.

An alternative approach for CRAD construction is control of the key genes with tissue- or tumour-specific promoters (TSPs). Promoters that have been utilised in this context include prostate-specific antigen, probasin, kallikrein and osteocalcin for prostate cancer, alpha-fetoprotein for hepatocarcinoma, DF3/MUC1 and pS2 for breast cancer, and many of these agents are undergoing clinical evaluation. Considering the highly variable CAR expression on human primary tumours, it seems likely that CRAD efficacy could be dramatically increased with the enhancement of infectivity. In pre-clinical models, it has been demonstrated that the oncolytic effect of CRADs directly correlates with infectivity [21,30]. Thus, a crucial determinant of the clinical usefulness of a CRAD is the balance between replicative potency and infectivity on the one hand and specificity on the other.

#### 5. Other cancer gene therapy vehicles

*Retroviruses* have been used extensively in gene therapy, but the results in randomised clinical trials for cancer have been disappointing due to inherent limitations of the vector [24,31]. High titre production of retroviruses is difficult and only dividing cells can be infected. However, there are strategies such as *ex vivo* transduction of leucocytes with drug resistance genes, for which retroviruses could be well suited. In addition, targeting of retroviruses and the creation of conditionally-replicating retroviruses could make this classic tool more useful in oncology. *Lentiviruses* are technically a subclass of retroviruses, but they can also infect non-replicating cells. However, high titre production is not easy and as most vectors are based on HIV, safety concerns need to be fully addressed before clinical applications can be considered.

*Herpes viruses* were among the first replication-competent viruses that were utilised for cancer treatment [32,33]. Pathogenicity is reduced by mutating one of the crucial virulence genes, resulting in replication competence only in cycling cells. Therefore, these agents are not truly tumour selective, but could be useful in the context of compartmental disease such as glioma. Unfortunately, current agents seem to possess relatively low oncolytic potential, and more sophisticated constructs may be necessary for adequate antitumour effects in humans.

There is no human disease known to be caused by *adeno-associated viruses* (AAV), but it can infect and integrate its genome into various types of human cells. Therefore, it presents an attractive candidate for applications where long-term gene expression is desired. In oncology, this could be useful with anti-angiogenesis strategies or other approaches where the purpose is not to kill the infected cells, but to use them to produce antitumour molecules. Polio, vaccinia and alphaviruses have been used as gene delivery platforms, but their role in oncology is currently not clearly established.

The concept of using *lipids* and other *non-viral agents* is attractive, as synthetic design and manufacture could produce less complex agents, which could have more predictable pharmacodynamics and -kinetics. However, this is also their limitation, as biological gene transfer vehicles (viruses) are the optimised products of evolution and it is hard for humans to produce similar efficiency in a few decades. An interesting field is the development of synthetic viruses, i.e. using components of viruses for improving the attributes of synthetic constructs. With increased understanding of the biological details of viral gene transfer, approaches combining components from different agents, biological or synthetic, could become increasingly popular.

## 6. Monoclonal antibodies

The use of monoclonal antibodies for the treatment of cancer is based on the assumption that tumours express certain moieties to a higher degree than normal cells. These epitopes could then be targeted with antibodies, with the antitumour effect produced by complement- or cell-mediated mechanisms. Alternatively, monoclonal antibodies can be conjugated with toxins, prodrug converting enzymes, or radioactive molecules. Recent clinical successes have validated the approach (Table 1).

## 7. Cancer vaccines

Cancers arise from human cells, but the plethora of mutations associated with the malignant phenotype also produces proteins that would be expected to be recog-

nised by the immune system. By definition, any growing cancer will have acquired immune tolerance for any heterologous epitopes. Cancer vaccination is based on the assumption that this tolerance can be broken with appropriate stimuli. Current strategies are usually based on one of two approaches. Either cancer cells are modified to secrete a stimulatory molecule (e.g. IL-2) so that they can be recognised by the immune system, or immune cells are modified to recognise tumour epitopes. Typically, the former is performed *ex vivo* and the latter can be done *in vivo* by transducing, e.g. cutaneous dendritic cells with tumour epitopes. A number of promising trials have been reported recently [34], and clinical applications could be imminent.

## 8. Small molecular inhibitors

Typically, small molecular inhibitors compete with molecules such as adenosine triphosphate (ATP) for binding sites and reduce activity of tumour-associated receptors or kinases (Table 1). The BCR/ABL fusion protein is the hallmark of chronic myeloid leukaemia, and treatment with STI571 resulted in complete haematological response in 53/54 patients [35]. Expression of c-kit characterises gastrointestinal stromal tumours, and STI571 results in dramatic responses [36].

## 9. Conclusions

The emergence of sophisticated tools for molecular and genetic analyses of cancers has created the concept of molecular-based treatment. A common denominator for the various strategies is detection of tumour-associated changes and subsequent treatment with the appropriate agents, often utilising combinations. Monoclonal antibodies and small molecular inhibitors have displayed clinical benefit and were quickly accepted for the treatment of patients. Gene therapy and cancer vaccines have displayed tremendous promise in trials and the final clinical breakthroughs are imminent. One of the crucial challenges will be realising the full potential of molecular-based agents in routine clinical practice. A requirement for rational molecular-based therapy must be the molecular analysis of tumours and consecutive informed decisions on which agents to use. There is little doubt that current developments are just the tip of the iceberg and thus the future of molecular-based therapies for cancer looks very exciting indeed.

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