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Novel approaches in oncology at AstraZeneca

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Abstract

Advances in the understanding of tumour biology have led to the discovery of new targets that control specific mechanisms essential for tumour spread, growth and survival. In order to fully explore the anticancer potential of these novel approaches, AstraZeneca is developing a broad pipeline of agents targeting a variety of key processes in tumour progression and metastasis. These include two novel antiangiogenic agents, ZD6474 and AZD2171, which are both orally available inhibitors of vascular endothelial growth factor receptor-2 tyrosine kinase, AZD2171 being a highly potent inhibitor; ZD6474 also has activity against epidermal growth factor receptor tyrosine kinase. Once-daily administration of these agents has been shown to result in effective inhibition of tumour growth in a broad spectrum of human xenograft models. In contrast to this approach, which prevents new vessel formation, the vascular-targeting agent ZD6126 disrupts the microtubular network responsible for maintaining the shape of immature endothelial cells, thereby selectively destroying the existing tumour vasculature and leading to extensive central necrosis. Other agents with a variety of novel antitumour strategies are also in development. These include AZD0530, an orally available Src kinase inhibitor, and AZD3409, an oral prenyl transferase inhibitor, both of which have potential for broad antitumour activity. In addition, an oral, selective, cyclin-dependent kinase inhibitor (AZD5438).

Keywords: Vascular targeting; Antiangiogenesis; ZD6474; ZD6126; AZD2171; AZD0530; AZD3409; AZD5438

1. Introduction

Increases in our knowledge of the mechanisms by which tumour cells become established, divide and metastasise has enabled the identification of a range of new targets, the inhibition of which might interfere with a variety of mechanisms essential for tumour growth and survival. In addition to targeting the tumour cells directly, the possibility of eliciting an indirect effect by destroying the tumour's blood supply presents an exciting new strategy with significant therapeutic potential. In contrast to conventional cytotoxic therapies, which generally target all proliferating cells, these novel molecular-targeted approaches exploit key differences between normal tissue and tumour tissue, or are directed against targets that are overexpressed or overactive in tumour cells. They could therefore act with a greater degree of specificity than cytotoxic chemotherapy.

In order to fully explore the anticancer properties of these novel approaches, AstraZeneca is developing a broad pipeline of agents targeting a variety of key processes in tumour progression and metastasis. A selection of these, at various stages of development, is outlined in Table 1.

2. Targeting the tumour vasculature

Agents that target the tumour vasculature act by limiting the blood supply to the tumour, and can therefore

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Table 1
Targeting key processes in tumour progression and metastasis

Agent	Description	Phase of development
ZD6474	Inhibitor of VEGF receptor tyrosine kinase with additional activity against EGFR tyrosine kinase	Phase II clinical
ZD6126	Vascular-targeting agent	Phase II clinical
AZD2171	Highly potent VEGF receptor tyrosine kinase inhibitor	Phase I clinical
AZD3409	Prenyl transferase inhibitor	Phase I clinical
AZD0530	Src kinase inhibitor	Preclinical
AZD5438	Selective cyclin-dependent kinase inhibitor	Preclinical

arrest tumour growth and limit metastatic spread. Such antivascular agents fall into two broad categories based on their general mechanism of action: antiangiogenic agents inhibit the angiogenic process and prevent new vessel formation while vascular-targeting agents directly target and destroy existing tumour vasculature.

2.1. ZD6474 and AZD2171: inhibition of angiogenesis

Angiogenesis refers to the formation of new blood vessels from pre-existing vasculature, a process that involves endothelial cell activation by pro-angiogenic factors and release of proteolytic enzymes, followed by endothelial cell migration, proliferation and capillary tube formation. Angiogenesis is essential for tumour growth beyond approximately 2–3 mm in diameter [1]. The vascular endothelial growth factor (VEGF) is crucial for blood vessel formation and plays a key role in endothelial cell survival signalling in newly formed vessels [2,3]. VEGF also induces significant vascular permeability, resulting in the highly permeable vessels that are characteristic of tumour vasculature [3–5]. Therefore, inhibition of VEGF signalling has considerable therapeutic potential. Technically, there are a number of different approaches to achieve inhibition of VEGF signalling, including sequestration of VEGF, blocking of VEGF binding to receptors, or inhibiting the tyrosine kinase activity of VEGF receptors (Fig. 1). ZD6474 is a novel inhibitor of VEGF receptor-2 (KDR) tyrosine kinase, with additional activity against epidermal growth factor receptor (EGFR) tyrosine kinase [6]. Chronic once-daily oral administration of ZD6474 has been shown to result in dose-dependent inhibition of tumour growth in a range of histologically distinct human xenograft models (breast, lung, prostate, colon, ovary and vulval; Fig. 2) as well as inducing profound tumour regression in established PC-3 prostate xenografts [7]. ZD6474 also demonstrates significant activity in orthotopically implanted tumour models and in models of metastasis [8,9]. This broad-spectrum activity is consistent with inhibition of VEGF signalling.

Phase I clinical evaluation of ZD6474 is now complete and preliminary reports of tumour regression have been documented in patients with non-small-cell lung cancer [10]. Adverse events were generally mild and included rash, diarrhoea and asymptomatic QTc prolongation. The anti-VEGF activity of ZD6474 has been suggested by a delay in dermal wound angiogenesis [11]. Clinical evaluation of ZD6474 continues in a series of phase II studies.

Other novel antiangiogenic agents include AZD2171, a highly potent inhibitor of VEGFR-2 tyrosine kinase activity and VEGF signalling, that lacks activity against EGFR tyrosine kinase. Once-daily oral administration of

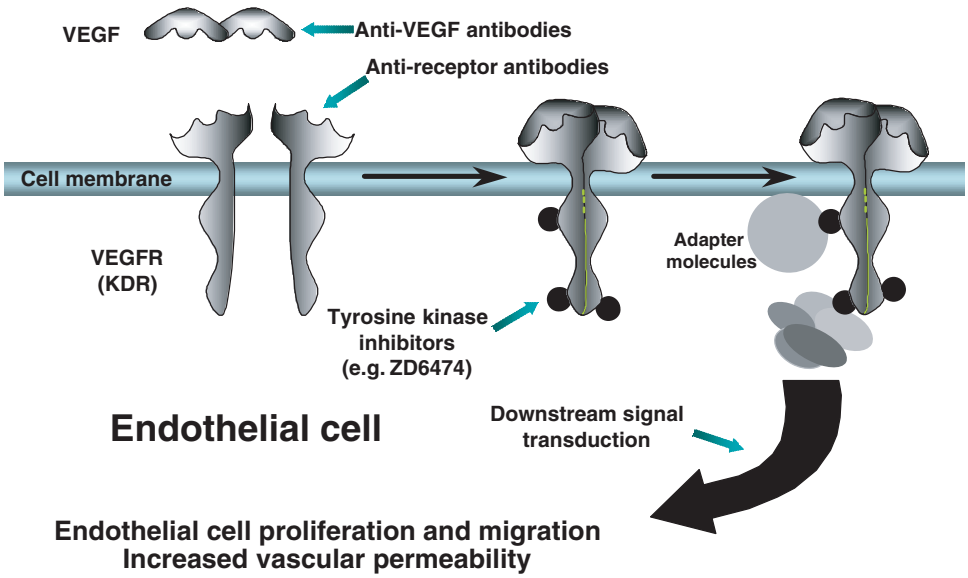

Fig. 1. Targeting VEGF receptor signalling.

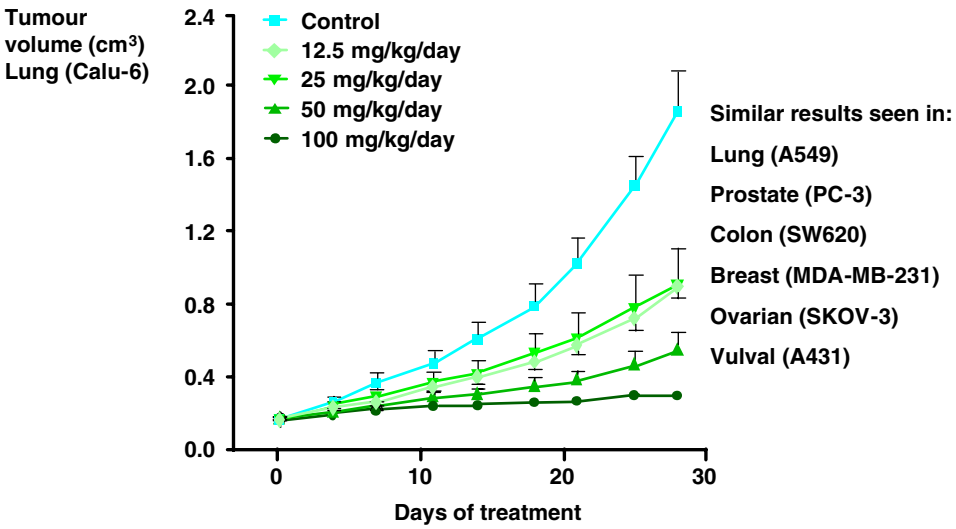

Fig. 2. ZD6474 inhibits growth in a range of tumour xenograft models [7].

AZD2171 also inhibits the growth of a broad spectrum of human xenograft models. This compound is currently in phase I clinical development.

2.2. ZD6126, a novel vascular-targeting agent

The vascular network that results from tumour angiogenesis differs greatly from normal blood vessels, comprising chaotic, highly tortuous, poorly formed, thin-walled vessels [12]. Permeability is unusually high due to the presence of large endothelial cell gaps, an incomplete basement membrane and a comparative absence of smooth-muscle cells [13]. Vascular-targeting agents exploit the structural and physiological differences to elicit selective destruction of tumour blood vessels. By targeting existing tumour vasculature, this approach has the potential to result in tumour necrosis and possibly regression.

ZD6126 is a novel vascular-targeting agent that disrupts the microtubular network responsible for maintaining the shape of immature endothelial cells. In proliferating endothelial cells, such as those of the tumour vasculature, this results in rapid morphological changes that lead to endothelial cell detachment and tumour vessel congestion (Fig. 3) [14]. In contrast, normal, mature endothelial cells are much less reliant on tubulin due to the presence of a well-defined actin cytoskeleton, interactions with

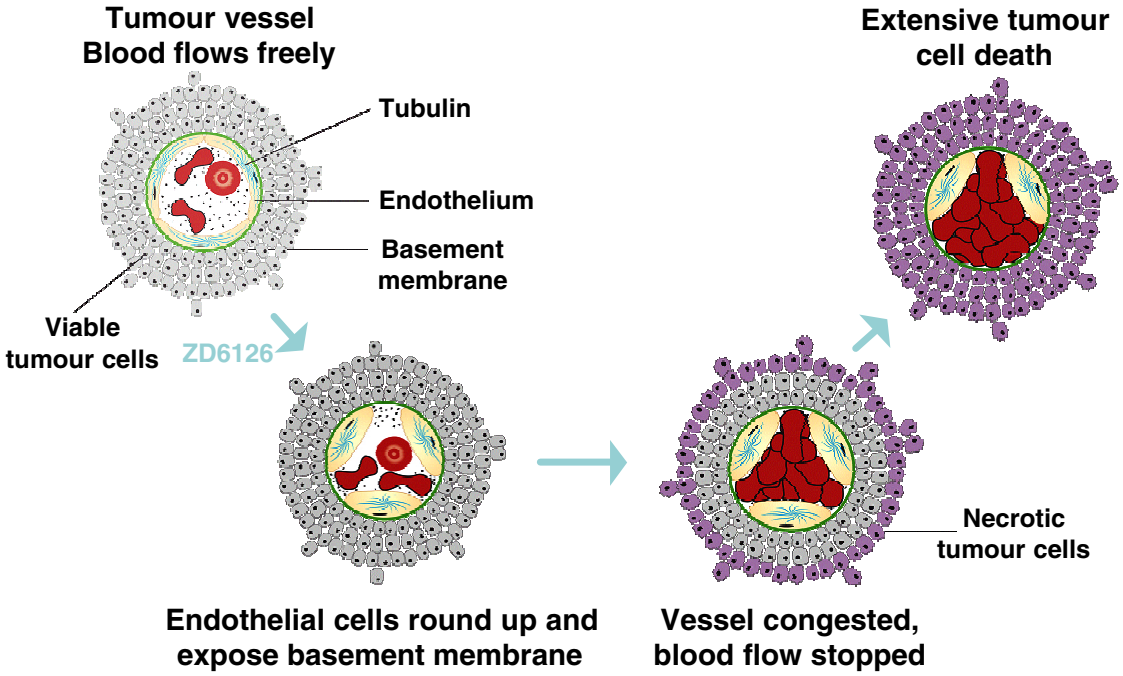

Fig. 3. ZD6126 mechanism of action.

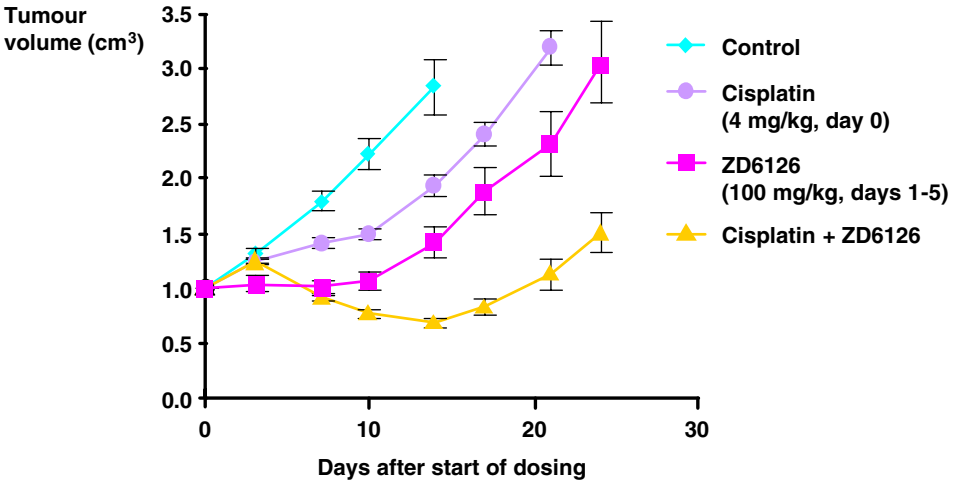

Fig. 4. Growth of Calu-6 human tumour xenografts after treatment with 4 mg/kg cisplatin i.p. and ZD6126 i.p. (100 mg/kg × 5 days) alone and in combination. The mean tumour volume of groups of 9–15 mice are shown [14].

a mature basement membrane and *in vivo* support by associated pericytes. Because of this, in the presence of ZD6126, normal, mature endothelial cell conformation is maintained [15]. ZD6126 treatment has been shown to induce selective disruption of tumour blood vessels resulting in extensive central necrosis in a range of tumour xenograft models [14]. At the periphery of the tumours, a thin rim of viable tumour tissue remains due to nutrient transfer from the surrounding normal vasculature. Combining ZD6126 therapy with certain conventional treatments such as cisplatin (Fig. 4) or radiation therapy can effectively target these remaining viable cells, improving on the therapeutic efficacy of ZD6126 alone [14,16,17]. A broad phase II programme is being progressed to assess the efficacy and tolerability of ZD6126 in monotherapy and combination regimens. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) has been used to visualise the activity of ZD6126, demonstrating a dose-dependent reduction in tumour perfusion [18].

3. Novel tumour cell targets

3.1. Src kinase inhibition

Members of the Src family of protein kinases are non-receptor, intracellular tyrosine kinases that initiate intracellular signal transduction pathways that influence migration, proliferation, adhesion and angiogenesis. Src kinases are highly regulated and expressed at low levels in most normal cells; however, in several human tumours, particularly colon and breast cancers, Src kinase is deregulated and its activity increased [19–22]. In colon tumours, Src kinase activity is frequently associated with the emergence of premalignant adenomatous polyps, which has been shown to increase with tumour progression and to be an indicator of poor prognosis [20,21,23]. In its active state, Src kinase is translocated to the cell periphery where initiation of intracellular signal transduction pathways leads to changes in adhesion strength [24]. Deregulation of Src kinase in cancer cells may, therefore, stimulate migration and promote an invasive cellular phenotype. Inhibition of Src kinase activity has been identified as a potential therapeutic target for a variety of tumour types.

The *in vivo* antitumour activity of a novel, orally available Src kinase inhibitor has been assessed in an orthotopic model of pancreatic cancer. In comparison with control animals, Src kinase inhibition resulted in a significant inhibition of primary tumour growth (approximately 40%). The most notable effect, however, was on metastatic spread, as Src kinase inhibition substantially reduced the development of liver metastases [25]. These studies are supported by *in vitro* data, demonstrating that this agent is a potent inhibitor of L3.6pl pancreatic tumour cell migration at submicromolar dose levels [25].

AZD0530 is one of a new anilinoquinazoline series of potent and selective Src kinase inhibitors. This orally available agent is currently in preclinical development as an anti-invasive agent and has potential for activity in a wide range of tumour types, particularly colon and breast cancers.

3.2. Inhibition of prenyl transferases

Prenylation is the post-translational modification of protein structure to add a lipid chain to the carboxy terminus. Prenylation facilitates the docking of proteins into cell membranes where they can be activated to perform their cellular function. A number of prenylated proteins specifically responsible for controlling intracellular signals required for cell division have been identified. Ras is one such protein: it plays a key role in signal transduction pathways that influence a number of impor-

tant biological processes such as cellular proliferation, apoptosis and cytoskeletal organisation. Prenylation of Ras proteins by the enzyme farnesyl transferase (FTase) causes the protein to localise on the inner surface of the cell membrane, where it exerts its biological effects [26]. In tumour cells, mutant forms of Ras result in constitutive activation of this pathway leading to uncontrolled cell division. It is known that, when farnesylation of Ras proteins is blocked, prenylation can occur via alternative pathways, thereby retaining some signal transduction capability [27].

AZD3409 is a novel oral prenyl transferase inhibitor, the active moiety of which has been shown to be a potent inhibitor of purified FTase. AZD3409 also has activity against geranylgeranyl transferase-1 (GGTase-1), another enzyme involved in protein prenylation, which may be relevant to its anticancer activity. In cultured mammalian cells, AZD3409 achieved up to 90% inhibition of FTase at well-tolerated doses [28]. Preclinical *in vivo* evaluation has shown that once-daily oral administration can provide 24-hour inhibition of FTase.

4. Cell cycle inhibition

In normal tissues, the cell cycle is tightly regulated to ensure an appropriate level of cell division and accurate DNA replication. Tumour cells, however, develop mechanisms to escape regulated growth control and replicate uncontrollably. A dramatic improvement in our understanding of cell cycle regulation has enabled identification of key differences in tumour cell processes, inhibition of which could be used to bring tumour replication under control. Novel cell cycle inhibitors target proteins that are deregulated in proliferating tumour cells; these differ from cytotoxic drugs, which act against generic cell targets and therefore not specifically on tumour cells.

4.1. Inhibition of cyclin-dependent kinases

Several members of the cyclin-dependent kinase (CDK) family regulate initiation, progression and completion of the normal cell cycle. For example, CDK1 regulates entry into and exit from mitosis; CDK4 and/or CDK6 drive progression through the G1 phase; and CDK2 plays a critical role in the entry into and progression through S phase and may also play a role in the G2/M phase transition [29]. Tumour cells develop mechanisms that overcome the usual cell cycle regulation, overexpressing cyclins and losing CDK inhibitor expression. Subsequent CDK deregulation therefore leads to uncontrolled cell growth.

A series of novel imidazopyridines that are selective CDK inhibitors have been generated and assessed for antitumour effect. These agents display a mechanism of action consistent with the induction of G1/S and G2/M phase arrest; once the cell cycle has been halted, apoptosis of tumour cells is seen [30]. In contrast, normal cells have the ability to recover from cell cycle arrest induced by these agents, offering the potential to elicit a differential effect between tumour and non-tumour tissues. AstraZeneca has identified selective CDK inhibitors, such as AZD5438, that show antitumour effects following oral dosing in preclinical *in vivo* studies. These agents have potential for the treatment of a wide range of proliferating tumours.

5. Conclusions

Cancer therapy continues to be an area of concentrated effort, with ongoing improvements in drugs and therapeutic regimens. While conventional cytotoxic interventions will continue to play a key role in the treatment of cancer, developments in novel targeted approaches generate considerable optimism that yet further advances can be made, providing agents that alone or in combination could prove to be of clinical benefit to patients with a wide range of tumour types. Using innovative translational science and biological markers of treatment effect, it is anticipated that the positive indications seen in preclinical evaluations can be assessed to best effect in the clinical environment, ensuring that the full potential of these novel therapies can be realised.

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