Tumor-vascular disrupting agents (VDAs) are a new class of anti-cancer drugs that show strong promise in treating a variety of solid tumors. Solid tumors include sarcomas, carcinomas, and lymphomas, and they account for more than 85% of cancers in humans.1 Whereas many approved treatments for solid tumors work by thwarting the formation of new blood vessels, VDAs attack a tumor’s existing vasculature, setting off a cascade of events that result in central tumor necrosis.2 ASA404 (DMXAA) is a novel VDA with a unique dual mechanism of action. ASA404 has a high degree of selectivity for tumor endothelial cells,3 and studies have found it is effective and well tolerated.

What is Angiogenesis?

Angiogenesis—the process by which tumors develop vasculature to deliver oxygen and nutrients to cells and remove metabolic waste—is an essential component of metastasis. To understand how VDAs work, it helps to understand angiogenesis.

Like normal cells, tumor cells need a consistent supply of oxygen and nutrients to survive and proliferate.4 Cells of small tumors (<2 cm) absorb oxygen from nearby healthy tissue via diffusion; this leaves tumor tissue perpetually hypoxic and limits tumor growth.5 Normal cells and tumor cells naturally produce anti-angiogenic factors, such as tumor necrosis factor alpha (TNFα), serotonin (5HT), nitric oxide (NO), thrombospondin, angiostatin, and endostatin,4 which also keep tumor growth in check.

Researchers have not determined exactly what causes a tumor to initiate angiogenesis, but they believe the “angiogenic switch” gets flipped to a perpetually on position, shifting the balance between angiogenic and antiangiogenic molecules. Hypoxia is believed to be one trigger. Hypoxia induces significant upregulation of vascular endothelial growth factor (VEGF) in cancer cells.6 Various oncoproteins, cellular receptors, cytokines, and other growth factors also signal VEGF expression.7 VEGF activates tyrosine kinase receptors on the surface of endothelial cells and is the most potent proangiogenic molecule involved in endothelial cell proliferation and blood vessel formation.4 Most solid tumors have been found to overexpress VEGF.

The tumor’s new vasculature provides malignant cells with a route for metastasization, as individual cells break off from the tumor and transit the network of capillaries to infiltrate distal organs.6 If the cancer cells are unable to establish a sufficient blood supply at the new location, they die or remain dormant. Dormant cells retain their cancerous properties and may trigger angiogenesis years after a primary tumor has entered remission.8
**Antiangiogenic Agents**

Since 1971, when Judah Folkman, MD, first hypothesized that tumor growth depended on angiogenesis, researchers have looked for ways to thwart the process. Early efforts focused on finding agents to inhibit this neovascularization. The US Food and Drug Administration has approved several monoclonal antibodies and tyrosine kinase inhibitors (TKIs) for this purpose, and others are in development. Approved antiangiogenic agents target circulating VEGF or inactivate specific VEGF receptors in an effort to prevent cancerous tumors from forming new blood vessels. They do not attack a tumor’s existing vasculature.

Antiangiogenic agents have limitations. They rarely produce complete tumor regression and are not curative. Malignant cells that survive treatment resume proliferation and neovascularization once the patient discontinues the drug. This necessitates months or years of chronic use, which can lead to drug resistance or adverse effects. Research suggests most patients become resistant to antiangiogenic agents after 6 to 12 months of continuous therapy.

In addition, solid tumors are often heterogeneous and use more than one signaling pathway to facilitate angiogenesis. Cells unaffected by the antiangiogenic agent continue signal transduction, resulting in relapse and possible distant metastases.

**Tumor-Vascular Disrupting Agents**

Unlike angiogenic agents, which prevent blood vessel formation, tumor-VDAs shut down the tumor’s established network of blood vessels. Total suppression of blood flow in the tumor causes ischemia in tumor tissue and necrosis at the tumor’s core. Complementary treatment with chemotherapy to target the viable rim of malignant cells remaining at the periphery of the tumor improves the likelihood of satisfactory results.

Several VDAs are in development and in trials, but none are approved for clinical use. Small-molecule VDAs such as ASA404, a low molecular weight antivascular agent, are the furthest along in clinical trials. ASA404 is the only VDA currently in phase III trials, and it has demonstrated highly promising results—even curative, in some cases—in mouse and human studies.

**How does ASA404 work?** ASA404, previously known as DMXAA (5,6-Dimethylxanthene-4-acetic acid), is an active analogue of flavone acetic acid. ASA404 has a dual mechanism of action that begins with exploiting weaknesses in the tumor’s immature blood vessels (Figure 1).

Tumor vasculature differs significantly from that of normal tissue. Blood vessels are tortuous, irregularly shaped, and leaky. The endothelial cells lining the vessels proliferate rapidly and have abnormal basement membranes. The vessels themselves demonstrate a dearth of pericytes (smooth muscle cells that envelop the vascular tube), and blood flow tends to be sluggish.

ASA404 acts directly on the tumor’s endothelial cells, and within minutes of intravenous administration, begins to induce endothelial apoptosis. The drug reorganizes the endothelial cells’ cytoskeletal network and disrupts cell-to-cell junctions, leaving the cells distorted. This exposes components of the basement membrane, causing platelets to aggregate in response to the damage. The platelets release serotonin (5HT), itself an antivascular agent, which the liver metabolizes into 5HIAA. Previous studies have found that plasma concentrations of 5HIAA constitute a useful measure of ASA404’s intravascular effects.

Within 2 to 3 hours of administration, ASA404 indirectly induces synthesis of TNFα in plasma and tumor tissue. After 6 hours, macrophages release NO and other cytokines that, in conjunction with TNFα, increase vascular permeability. This results in plasma leakage. The loss of plasma increases blood viscosity and restricts the diameter of the capillaries, decreasing blood flow throughout the tumor. With blood flow restricted, the ASA404-induced cytokines and antivas-

![Figure 1. ASA404 Mechanism of Action](image-url)
cular agents are trapped in the tumor, enhancing their antiangiogenic effects.2

Downstream hemorrhagic necrosis depends on complete cessation of blood flow in the tumor long enough to deplete cellular energy reserves. Between 12 and 24 hours after administering ASA404, blood vessels rupture and erythrocytes extravasate into surrounding tissue. Approximately 70 minutes after blood flow has ceased, apoptosis escalates rapidly, leading to extensive tumor necrosis at the tumor’s core.2 A layer of cells at the tumor’s periphery survives, possibly sustained by absorbing oxygen and nutrients from unaffected normal tissue nearby. Unless another treatment, such as a cytotoxic agent, is used to destroy these cells, they will quickly repopulate the tumor.

Researchers have hypothesized that patients are less likely to develop drug resistance to VDAs like ASA404. This is because ASA404 targets endothelial cells, which have greater genetic stability than neoplastic cells.4

**Phase II studies of ASA404.** Added to standard chemotherapy with carboplatin and paclitaxel (CP), ASA404 has demonstrated excellent efficacy as a first-line treatment for patients with squamous and nonsquamous non–small cell lung cancer (NSCLC).13 A phase II study randomized 70 patients with untreated stage IIIb/IV NSCLC to receive ASA404 1200 mg/m² plus ≤6 cycles of chemotherapy with carboplatin (AUC 6 mg/ml/m) and paclitaxel (175 mg/m²) or CP alone. In a follow-up single-arm extension study, 30 patients received ASA404 1800 mg/m² combined with the CP regimen used in the previous study. Patients in the 3 treatment groups were well matched according to age, sex, and cancer stage; approximately 30% of patients in each arm had NSCLC of squamous histology, a reflection of its incidence in the general population.

Investigators pooled data from both studies to compare the safety and efficacy of ASA404 plus CP versus CP alone and to determine whether adverse events or treatment outcomes differed between patients of squamous and nonsquamous histology receiving identical treatment. Primary outcome measures included response rate, median time to tumor progression, and median survival. ASA404 plus CP was associated with superior efficacy across the board (Table), extending median overall survival an average of 4 to 5 months compared with CP alone (14.5 m vs 8.8 m, respectively). Response rate with ASA404 plus CP was 34.4% versus 22.2% for CP alone.13

Squamous patients in the CP-only group had less favorable outcomes than nonsquamous patients across all end points. In the combination group, investigators found no consistent differences in outcomes between squamous and nonsquamous patients but noted that squamous patients in all treatment arms had shorter median survival.

Across all patient subgroups, the most common adverse events were those typically associated with chemotherapy. Similar proportions of patients in each treatment group experienced at least one adverse event ≥grade 3. Patients receiving ASA404 plus CP had a higher incidence of anemia and neutropenia, and squamous patients receiving combined treatment were more likely to experience thrombocytopenia.

Although patients taking ASA404 plus CP reported more cardiac adverse events, no vascular events were ≥grade 3 in patients with either histology. Investigators noted that a phase II study of the antiangiogenic monoclonal antibody bevacizumab found that a higher proportion of patients with squamous NSCLC experienced life-threatening pulmonary hemorrhage with this agent compared with nonsquamous patients (31%...
vs 4%, respectively). ASA404 plus CP appeared to be well tolerated (Figure 2); researchers noted that hematological toxicities were increased but manageable.13

**Phase III studies of ASA404.** ASA404 has entered two phase III multinational, randomized, double-blind, placebo-controlled studies. ATTRACT (Antivascular Targeted Therapy: Researching ASA404 in Cancer Treatment)-1 is investigating ASA404 1800 mg/m² in combination with CP as a first-line treatment for NSCLC. Researchers are enrolling 1200 patients with untreated locally advanced or metastatic NSCLC of squamous and nonsquamous histology.

ATTRACT-2 is enrolling 900 patients with stage IIIb/IV NSCLC who have already undergone first-line treatment with chemotherapy plus bevacizumab or cetuximab. Patients will be randomly assigned to receive either ASA404 1800 mg/m² or placebo plus docetaxel. The primary end point for both trials is overall survival; results are expected in 2011, with interim analyses presented in 2010.

**Conclusions**

Tumor VDAs are an exciting investigational class of therapeutic agents that may open up new avenues of treatment for patients with solid tumors. ASA404, the only VDA in phase III clinical studies, has demonstrated remarkable efficacy in patients with squamous and nonsquamous NSCLC. In addition to studies involving patients with lung cancer, investigators are examining ASA404 as a potential treatment for other solid tumors and in combination with other therapeutic agents.2

**References**


---

**Figure 2.** Most Common Adverse Events ≥Grade 3 in Patients Receiving ASA404

<table>
<thead>
<tr>
<th>Adverse Events</th>
<th>Percent (%)</th>
<th>Squamous</th>
<th>Nonsquamous</th>
<th>All Histologies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood/Lymphatic</td>
<td>18</td>
<td>22</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Nervous System</td>
<td>14</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Skin/Subcutaneous</td>
<td>14</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Infections/Infestations</td>
<td>18</td>
<td>11</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>General/Admin Site</td>
<td>9</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Any ≥Grade 3</td>
<td>68</td>
<td>61</td>
<td>63</td>
<td></td>
</tr>
</tbody>
</table>

---

**Legend:**
- Squamous
- Nonsquamous
- All Histologies