

## Review of VDA

There are two types of vascular disrupting agent (VDA). First, biological or ligand-directed VDAs use antibodies, peptides or growth factors to target toxins or pro-coagulants to the tumor endothelium. In contrast, small molecule VDAs work either as tubulin-binding agents or through induction of local cytokine production.<sup>1</sup> Microtubule depolymerizing agents form by far the largest group of small molecular weight VDAs. The combrestatins, including the lead compound disodium combretastatin A-4 3-O-phosphate (CA4P), are structurally related to the classical tubulin-binding agent colchicine and are themselves tubulin binding, microtubule depolymerizing agents.<sup>2</sup> 5,6-dimethylxanthenone-4 acetic acid (Antisoma's ASA404; previously known as DMXAA) and flavone acetic acid (FAA) are two of a series of drugs that have several antivasular actions, including the induction of cytokines. The VDAs that are currently used produce a much greater blood flow reduction in tumors than in normal tissues<sup>3-5</sup> and this forms the basis for their acceptance into clinical trials. Indeed, both CA4P and ASA404 are being evaluated in Phase II clinical trials in combination with conventional cytotoxic therapies for the potential treatment of cancer.<sup>6</sup> In the first Phase I and II trials of VDAs, the toxicity profile was unlike that of conventional chemotherapy, which is encouraging for combined therapeutic approaches. Although generally well tolerated, dose limiting toxicities of CA4P included chest pain and/or cardiotoxicity, reversible ataxia, vasovagal syncope and motor neuropathy.<sup>7-9</sup> The most common toxicities of ASA404 included reversible confusion, tremor, slurred speech and visual disturbance.<sup>10,11</sup> Thus there is still an unmet need for VDAs with more favorable safety profiles and fewer side effects.

The susceptibility of tumor vessels to VDA damage is ascribed to their immature pericyte-defective nature, although the exact molecular mechanisms involved have not been clearly defined. The tumor vasculature differs from the normal vasculature in a number of ways. The walls of tumor blood vessels are poorly developed, often with a discontinuous endothelial cell lining, have relatively poor investiture with vascular smooth muscle cells, and have poor connections between pericytes and endothelial cells.<sup>12-14</sup> These features contribute to a high intrinsic vascular permeability to macromolecules<sup>15,16</sup> and the consequent development of high interstitial fluid pressure.<sup>17</sup> Clearly, a further increase in permeability following VDA treatment is likely to be catastrophic to the tumor under these conditions. Another hallmark of the VDAs such as the combrestatins is the induction of a rapid collapse in tumor blood flow, often first detected within 5 minutes of drug treatment in animal models and leading to complete vascular shutdown by 20 minutes.<sup>18</sup> Tumor imaging studies have identified similar effects in Phase I clinical trials of these agents.<sup>19-21</sup> Drug-induced effects on endothelial cell proliferation and/or endothelial cell apoptosis occur too slowly to account for these antivasular actions *in vivo*; rather, morphological and functional changes in endothelial cells are more likely to cause the tumor vasculature collapse.<sup>2,22</sup> In addition to these primary effects, the chaotic organization of tumor blood vessels, which generally contain numerous crosslinking capillaries,<sup>23</sup> leads to reduced circulatory efficiency and in many cases can result in regional failure in response to decreased flow rates.<sup>2</sup> In addition, when blood flow falls below a certain level, red cells stack together to form rouleaux, increasing viscous resistance to flow and further blood stagnation.<sup>24</sup>

Preclinical data indicate that VDAs can improve the tumor response to cytotoxic chemotherapy, radiation and antiangiogenic treatments. This activity has been attributed to the ability of these agents to selectively destroy the central regions of tumors, areas widely believed to contain cell populations resistant to cytotoxic therapies.<sup>6</sup> However, despite causing profound damage to tumors, VDAs fail to halt tumor growth unless used together with conventional treatments. This failure is attributed to resistance mechanisms, primarily associated with cells that remain viable within the tumor rim of even the most responsive tumors. The viable rim explains the rapid repopulation of tumors after treatment and the consequent failure to achieve significant growth delay.<sup>25</sup> A common explanation for the viable rim is that tumor cells in that region survive because they receive oxygen and nutrients from the surrounding normal tissue. This implies that vascular damage in the periphery is the same as in the center but that the tumor cells in the outer region survive through their proximity to the undamaged surrounding normal tissue blood vessels.<sup>2</sup> However, there is also evidence to suggest that blood flow itself is less affected at the periphery than at the center following VDA treatment and this differential effect on blood vessels in different tumor regions needs to be explained or, at the very least, overcome. The extensive ischemic insult to tumors following VDA treatment results in pronounced tumor cell hypoxia even in the surviving viable rim.<sup>26,27</sup> This raises the

possibility of hypoxia-induced angiogenesis and the important clinical implication of this selective pressure in producing a more aggressive phenotype in the recurrent tumor.

Finally, the VDA, ASA404 (being developed by Antisoma plc) has demonstrated clinical efficacy in patients with advanced, hormone-refractory, metastatic prostate cancer when administered in combination with docetaxel (results from earlier, Phase I evaluation showed that ASA404 monotherapy was well tolerated).<sup>28</sup> Briefly, subjects were randomized to receive either 1200 mg/m<sup>2</sup> of ASA404 plus 75 mg/m<sup>2</sup> of docetaxel, or docetaxel alone. Addition of ASA404 was generally well tolerated. Prostate specific antigen (PSA) response rates were 59% with ASA404 plus docetaxel and 37% with docetaxel alone. Armstrong *et al.* suggests that the PSA measure most predictive of survival is the proportion of patients showing a 30% decline in PSA levels in the 3 months after the start of treatment.<sup>29</sup> Proportions of ASA404 patients with such a decline were 63% with ASA404 plus docetaxel and 47% with docetaxel alone, predicting that survival might be greater in the ASA404 arm. Indeed, Antisoma subsequently released results showing improved 2-year survival on this study (33% with ASA404 and 23% with docetaxel alone).<sup>30</sup> Moreover, tumor response rates in patients accessible by Response Evaluation Criteria in Solid Tumors (RECIST) were 63% with ASA404 plus docetaxel and 47% with docetaxel alone, and time to disease progression was 7.3 months (vs 6.9 months) according to investigators' assessment. Taken together, ASA404 results in advanced prostate cancer support the clinical potential of a novel-mechanism VDA such as STA-9584, and suggest that careful monitoring of existing, validated clinical biomarker data (namely PSA at 3 months' treatment) may be predictive of overall patient survival.

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