Imaging Tumor Sensitivity to a Bioreductive Prodrug: Two for the Price of One!

J. Martin Brown

Hypoxia is an important characteristic of many solid tumors and has a major negative effect on treatment response. A way to combat this effect is with drugs called "bioreductive prodrugs" or "hypoxic cytotoxins," which are metabolized under hypoxia to toxic species. However, the patients with hypoxic tumors need to be identified. Clin Cancer Res; 18(6): 1–3. ©2012 AACR.

In this issue of Clinical Cancer Research, Wang and colleagues (1) report that the hypoxia marker EF5 predicts for the potential antitumor activity of the bioreductive hypoxia-activated prodrug CEN-209, not only by detecting tumor hypoxia (low oxygen levels) but also by assessing the activity of the enzymes responsible for reducing the prodrug to its cytotoxic product under hypoxia.

For more than 60 years, the holy grail of research in radiation oncology has been a method to overcome the problem caused by the resistance of the hypoxic cells in tumors to killing by radiation. Many strategies have been tried, such as increasing tumor oxygen levels by patients breathing pure oxygen; using densely ionizing radiation such as neutrons, whose killing of cells is less dependent on oxygen; and the use of small molecules ("hypoxic cell radiosensitizers") that substitute for oxygen in the free radical reaction that makes permanent the radiation-induced radical damage to DNA. Through each of these strategies only imperfectly combats the hypoxia problem, the sum total of the clinical trials shows that modifiers of tumor hypoxia produce a highly significant, though modest, improvement in local tumor control and patient survival when combined with radiotherapy (2). However, perhaps the most promising strategy in recent years has been the development of agents that can actually exploit the fact that tumors are usually more hypoxic than normal tissues by selectively killing the hypoxic tumor cells (3). One such drug, a benzotriazine di-N-oxide, tirapazamine (TPZ), has a very high selectivity for killing hypoxic cells (with drug levels needed to kill hypoxic cells typically only 1% or less of those needed to kill aerobic cells) and has been tested in several clinical trials. However, the recent report of a large randomized multicenter phase III trial of TPZ combined with radiotherapy for head and neck cancer reported no benefit (4), although part of this could be attributed to major deficiencies in treatment in a subset of patients (5). Meanwhile, further drug development by the team led by Wilson and Hay in Auckland, New Zealand, has identified analogs of TPZ that are less toxic and that are superior to TPZ in penetrating tumor tissue, thereby providing greater benefit when combined with irradiation in preclinical studies (6). The analog SN30000 has emerged from these studies as the most promising and has gone into clinical development under the name CEN-209. So a very potent "son of tirapazamine," CEN-209, will hopefully soon be available for widespread clinical testing.

But, in addition to an active agent to overcome, or to exploit, tumor hypoxia, a critical need, especially in clinical trials, is a method of selecting the patients with the most hypoxic tumors, as only these patients will benefit from the addition of the agent. The lack of such a method was one of the shortcomings of the above-mentioned phase III trial of TPZ, as was clear from a subset of an earlier clinical trial of TPZ with radiotherapy in patients who had tumors that were identified as hypoxic or not (7). Ideally, a method to select hypoxic tumors would be noninvasive, and several positron emission tomography (PET) imaging agents are being developed for this purpose. These agents all involve the hypoxia-dependent reduction of nitroaromatic compounds typically containing the PET tracer 18F, and 18F-EF5 is one of the leading contenders with clinical studies showing its potential use (8). However, reduction of EF5 and the other nitroaromatic PET tracers to their reactive forms that bind irreversibly to the hypoxic cells requires not just low oxygen but also the activity of nitroreducing enzymes, some of which are yet to be identified. Thus, the PET signal from tumors from 18F-EF5 and other hypoxia tracers is dependent on the activity of the reductive enzymes in addition to the level of hypoxia.

How does this affect the sensitivity of tumors to bioreductive prodrugs such as CEN-209? All such drugs need bioreductive enzymes in addition to hypoxia, but again, although we know the identity of some of these, including NADPH:cytochrome P450 oxidoreductase (CYPOR), we do not know the identities of all. What Wang and colleagues set
out to address was whether there was a relationship between the activity of the enzymes that reduce EF5 to its hypoxia-binding species and those that reduce CEN-209 to its cytotoxic species. Although both require the addition of an electron from the reducing enzymes, there is no a priori reason to suppose that the same enzymes would reduce EF5 and CEN-209: The compounds are dissimilar in structure (a nitroaromatic and N-oxide, respectively) and in the severity of hypoxia required for their activation to their respective active metabolites. Yet, Wang and colleagues, using a battery of sensitive assays, which they did with great care with multiple important controls, found a very close correlation over a wide range of enzymatic activity between the reduction of EF5 and that of CEN-209, as well as cytotoxicity and DNA damage by CEN-209, under hypoxic conditions. Importantly, although they showed that the reducing enzyme CYPOR metabolized EF5 and CEN-209 to a similar extent, they showed that CEN-209 metabolism was more closely correlated with EF5 binding than with CYPOR activity, implying the presence of additional (as yet unknown) enzymes responsible for the reduction of both EF5 and CEN-209. This finding shows that EF5 binding (and, hence, strength of 18F-EF5 signal in tumors) provides a superior assessment of reductive metabolism (and, hence, cytotoxicity) of CEN-209 than does the activity of CYPOR or any other known enzymes.

These findings have important implications for the clinical use of CEN-209. As the drug kills only cells under hypoxic conditions, it is accepted that no clinical trials should be conducted without first selecting the patients with hypoxic tumors. There is a plethora of potential ways to do this, including directly measuring oxygen levels with electrodes, using immunohistochemistry of hypoxia-activated proteins such as CA9 or GLUT1, and PET imaging with nitroaromatic compounds such as 18F-EF5. However, the data presented by Wang and colleagues show that detecting hypoxia with EF5 has the major advantage over the other methods, in that it not only detects hypoxia but also assesses the level of prodrug-activating reductive enzymes needed to metabolize CEN-209 to its cytotoxic species. Thus, a tumor that “lights up” with 18F-EF5 would predict a high level of cell kill by CEN-209, whereas one that might be hypoxic but shows little 18F-EF5 activity would not be expected to be sensitive to the drug (Fig. 1). In effect, EF5 potentially images tumor sensitivity to CEN-209 by simultaneously assessing tumor hypoxia and the level of reductive enzymes. It is two for the price of one.

Disclosure of Potential Conflicts of Interest
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