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

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Running Title: Imaging tumor drug sensitivity
Summary: Hypoxia is an important characteristic of many solid tumors and has a major negative effect on treatment response. A way to combat this is with drugs, called “bioreductive prodrugs”, or “hypoxic cytotoxins”, that are metabolized under hypoxia to toxic species. However the patients with hypoxic tumors need to be identified.

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 over 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. Though each of these strategies only imperfectly combats the hypoxia problem, the sum total of the clinical trials demonstrates 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), though 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, NZ, 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 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 is clear from a subset of an earlier clinical trial of TPZ with radiotherapy in patients that had tumors that were identified as hypoxic or not (7). Ideally a method to select hypoxic tumors would be non-invasive and several PET imaging agents are being developed for this purpose. These all involve the hypoxia dependent reduction of nitroaromatic compounds typically containing the PET tracer $^{18}$F, and $^{18}$F-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 $^{18}$F-EF5, and other hypoxia tracers, is dependent on the activity of the reductive enzymes in addition to the level of hypoxia.

How does this impact the sensitivity of tumors to bioreductive prodrugs such as CEN-209? All such drugs also need bioreductive enzymes in addition to hypoxia but again though we know the identity of some of these, including NADPH:cytochrome P450 oxidoreductase (CYPOR), the identity of all are not known. 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. Though 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 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 performed with great care with multiple important controls, found a very close correlation over a wide range on 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 though they demonstrated 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 demonstrates that EF5 binding (and hence strength of $^{18}$F-EF5 signal in tumors) provides a superior assessment of reductive metabolism (and hence cytotoxicity) of CEN-209 than 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 $^{18}$F-EF5. However, the data presented by Wang et al 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 $^{18}$F-EF5 should be sensitive to CEN-209 whereas one that might be hypoxic, but shows little $^{18}$F-EF5 activity, would not be expected to be sensitive to the drug (Fig 1). In effect, EF5 is potentially imaging tumor sensitivity to CEN-209 by simultaneously assessing tumor hypoxia and the level of reductive enzymes. It is two for the price of one.
Fig 1: A schematic representation showing that the metabolism of the imaging agent 18F-EF5 to "light up" a tumor and the metabolism of the bioreductive prodrug CEN-209 to its cytotoxic species are both determined by the combination of tumor hypoxia and the same reductive enzymes, including CYPOR and others. Shown also are 18F-FDF and 18F-EF5 images of two head and neck tumors from patients to illustrate the fact that similar FDG images may not yield similar EF5 images, presumably because of differences in tumor hypoxia. Because a high 18F-EF5 signal would predict a high level of cell kill by CEN-209, the prediction would be that the tumor on the left would be sensitive whereas that on the right would be resistant to CEN-209. (The 18F-FDF and 18F-EF5 images are reprinted by permission of the Society of Nuclear Medicine from Komar G, et al. (8), Figure 4.)
Tumor hypoxia

Reductive enzymes: CYPOR and others

[18F]FDG

CEN-209

Sensitive Tumor

CEN-209 Insensitive Tumor

[18F]EF5

Tumor hypoxia

CEN-209
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