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Drug cocktails for effective treatment of glioblastoma multiforme

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“Just about everything that relates to glioblastoma multiforme pathobiology and its clinical course invites thinking about specific targeting of more than one tumor compartment/target and more than one mechanism controlling the pathobiology...”

Glioblastoma multiforme (GBM) is the most prevalent primary brain tumor and also one of the most difficult human malignancies to manage. In fact, only 7 months was added to the mean survival rate of patients with GBM during the last seven decades [1,2]. The location of tumor, which impacts on poor penetration of drugs through the blood–brain barrier and also within the tumor; well-known resistance to chemotherapy and radiation therapy also in part because of the location; the infiltrative nature reflected by tumor cells migrating away from the primary site and embedding into normal brain parenchyma; genetic, morphological and molecular heterogeneity; and extremely well developed although improperly working neovasculature all contribute to a dismal outcome of GBM patients. Recent years have brought more interest in studying GBM among cancer researchers generating new invaluable knowledge. This new knowledge at genetic, morphological, cellular and molecular levels provides a basis for rational drug development approaches. The prospect of finding better treatments for GBM is now certainly more realistic.

Just about everything that relates to GBM pathobiology and its clinical course invites thinking about specific targeting of more than one tumor compartment/target and more than one mechanism controlling the pathobiology of GBM, hence its maintenance and progression. This truly prompts researchers to consider combinatorial therapy or a cocktail of drugs. The current standard of care itself is a combination of different

modalities of treatment, such as surgery, chemotherapy and radiation, but they do not address fully, as they cannot, the formidable complexity of GBM. In this editorial, it is argued that combinatorial treatment can and should be applied utilizing various classes of drugs that are directed against different tumoral compartments and different aspects of cellular/molecular composition and pathobiological importance. A combinatorial (i.e., a cocktail) drug approach would be relevant within a specific group of drugs as well as among them. This would imply that perhaps not only a cocktail of drugs (nowadays rationally designed) against specific tumor compartments/functions will be needed, but also such a cocktail will need to include cocktails from each specific type of agents.

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A novel approach to GBM treatment is the use of molecularly targeted recombinant cytotoxins. Recombinant cytotoxins are targeted to GBM tumor cells by the means of a ligand or antibody that binds an internalized plasma membrane receptor. They deliver modified bacterial toxins as active, catalytic portions of the cytotoxins [3]. Several cytotoxins have been in clinical trials during the last decade or so even though none of them were designed for the treatment of brain tumors [3]. The most striking preclinical and clinical

results were obtained with a cytotoxin composed of a wild-type IL-13 and PE38QQR derivative of *Pseudomonas* exotoxin A [4]. An uncommon efficacy seen in patients with recurrent GBM in Phase I and II trials with IL13-PE38QQR [5] prompted development of Phase III trial that ended in late 2006. The drug demonstrated significantly better progression-free survival in patients receiving the cytotoxin versus current standard of care [6]. This efficacy was obtained even though intratumoral drug delivery through convection-enhanced delivery was not monitored, and patients were not selected for the overexpression of one of the drug's targets, IL-13 receptor- $\alpha 2$ (IL-13R $\alpha 2$). Here is the first reason why we should think about a cocktail of cytotoxins rather than an individual one. IL-13R $\alpha 2$ is overexpressed in approximately 75% of patients with GBM [7–10] although its gene is expressed at lower levels [11], not an uncommon scenario. Thus, at least a quarter, and perhaps even more than that, of patients with GBM are expected to be poor or nonresponders to therapies targeting IL-13R $\alpha 2$. This calls for prescreening patients before applying therapy, but then a sizable subset of patients will not have a chance to receive a drug based on a promising therapeutic strategy. One can envision that addition of another cytotoxin would make a difference. The prerequisite would be that there is another target in GBM that is not completely overlapping with the expression of IL-13R $\alpha 2$ and its distribution. EphA2 tyrosine kinase receptor is the second receptor that is highly overexpressed in patients with GBM and its expression is linked to a worse outcome [12,13]. A vast majority of GBM patients overexpress either IL-13R $\alpha 2$ or EphA2, but importantly approximately 95% overexpress two receptors [10]. Hence, a very small fraction of patients would be potential nonresponders if a cocktail of two cytotoxins, the ones targeting IL-13R $\alpha 2$ and EphA2, was administered. A prototype cytotoxin against EphA2 receptor has already been generated and tested [14]. In addition, a combination of two cytotoxins or double-targeting in GBM treatment makes the cytotoxins somehow more effective antitumor agents [15,16].

An ideal scenario would be to have a cocktail of anti-GBM cytotoxins targeted against receptors that are overexpressed in 100% of patients at any given time. The third receptor, besides IL-13R $\alpha 2$ and EphA2, highly overexpressed in GBM but not normal brain is currently being searched for as a suitable candidate for the development of another specific and potent cytotoxin. It is likely that we will identify such a target, since a protein, a transcription factor, the overexpression of which complements that of IL-13R $\alpha 2$ and EphA2, has been found. Fos-related antigen 1, together with IL-13R $\alpha 2$ and EphA2 constitutes the first trimolecular signature of GBM identified as such [10]. A combination of drugs targeting this trio of factors will not miss a single patient with GBM, but obviously a cytotoxin cannot be generated against Fra-1.

It is now possible to specifically target a neovasculature (vessels formed during tumor initiation and progression) of GBM with drugs. Clinical trials demonstrate that such a treatment

tightens up neovasculature and tumor vessels become even less permeable [17]. One could consider intratumoral delivery of recombinant cytotoxins under such conditions in expectation of longer residence time within the tumor and its vicinity with less leakage into the periphery. This requires having cytotoxins of neutral activity towards normal brain, such as those targeted against IL-13R $\alpha 2$ and EphA2. Clinical examination of a cocktail of cytotoxins/antiangiogens is warranted.

We hypothesized that GBM is powered, like a sports car, by multiple engine cylinders and it might be necessary to knock down most of them in order to effectively stop or at least weaken it [18]. Biological forces moving this engine are the intracellular signaling pathways. Recent extensive research in this area of investigation fully supports the notion that an Achilles heel of GBM has not yet been identified, but rather multiple complex mechanisms operating in GBM that maintain the tumor and cause it to progress [19]. Thus, inhibiting one activated system may slow the work temporarily but will not stop the engine. Preferably, two or three pathways should be attacked concomitantly or consecutively. This is another example of combinatorial therapy in which a cocktail of intracellular pathway inhibitors might need to be employed in the treatment of GBM.

“An ideal scenario would be to have a cocktail of anti-GBM cytotoxins targeted against receptors that are overexpressed in 100% of patients at any given time.”

And finally, I would like to make a case for having drugs for the treatment of GBM available off-the-shelf. Our current understanding of the disease and current ability to generate therapeutic compounds makes combinatorial therapy an approach of choice. The next decade or so of research will indicate which combinations/cocktails of drugs belonging to one group of compounds, such as small molecule inhibitors or recombinant cytotoxins, will work most effectively in patients. During this process, combining a cocktail of recombinant cytotoxins with a cocktail of small molecule inhibitors may make therapeutic sense in that the cytotoxins will eliminate as many tumor cells as they can, but even a residual disease will ascertain the recurrence, and hence needs to be taken care of. Small molecule inhibitors could then be introduced into the treatment regimen. Or, a multivalent vaccine following the cytotoxins/inhibitors will be necessary to be combined with the preceding treatment [20]. This is again in order to eliminate surgically inaccessible, chemotherapy-resistant/inaccessible and radiation-resistant/inaccessible tumors. The cost of developing off-the-shelf cocktails might actually be less than making individualized target identification for choosing an individual patient-profiled cocktail, and certainly more applicable in parts of the world where individualized medicine will not have economic support for a long time to come.

Financial & competing interests disclosure

Dr Waldemar Debinski is a consulting scientific advisor and a shareholder in Targepeutics, Inc. Dr Debinski is an inventor on the patent applications submitted or issued, which are also on the subject of this paper (however, Penn State University and Wake Forest University own the patents).

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