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CANCER

Turning glioblastoma cells vacuous

> vacquinol-1 was the most effective at reducing tumour size

Glioblastoma multiforme is notoriously difficult to treat; even with a triple-pronged approach including surgical resection, radiation and chemotherapy, less than 5% of patients survive for more than 5 years after diagnosis. In a new paper published in *Cell*, Kitambi and colleagues have identified a series of chemical analogues, which they call vacquinols, that kill glioblastoma cells while sparing normal cells.

Kitambi *et al.* used two primary cell cultures obtained from patients with glioblastoma to screen a US National Institutes of Health (NIH) compound library for chemicals that could induce cell death. To identify which of these compounds might be most therapeutically relevant, they whittled the 1,364 hits identified in their primary screen down to 17 by selecting compounds with no toxicity in murine embryonic stem cells, human fibroblasts, zebrafish embryos or adult zebrafish hearts treated *ex vivo*. These 17 hits were tested for *in vivo* efficacy in a zebrafish xenograft model in which one of the human glioblastoma cell lines used for screening was injected intracranially into zebrafish larvae; vacquinol-1 was the most effective at reducing tumour size.


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Vacquinol-1 does not induce cell death through apoptosis or classic autophagy-associated cell death. Rather, the authors found that it initiates macropinocytosis and the formation of large, single-membrane-encapsulated vacuoles inside the cell. Although the mechanisms through which this occurs and subsequently causes cell death are unclear, the authors hypothesize that contributing factors include the energy required to form these vacuoles and the increased hydrostatic pressure that results from having less cytoplasmic membrane. The dual specificity mitogen-activated protein kinase kinase 4 (MAPKK4), which activates the JUN N-terminal kinase (JNK) and p38 MAPK stress signalling pathways, probably has a role too: in a short hairpin RNA (shRNA) screen, targeting the gene encoding MAPKK4 rendered glioblastoma cells resistant to vacquinol-1-induced cell death.

In pharmacokinetic studies, the authors observed that vacquinol-1 is membrane-permeable, metabolically stable in liver microsomes (suggesting that it will not be rapidly degraded *in vivo*) and can penetrate the blood–brain barrier following intravenous administration. These observations together imply that vacquinol-1 could be orally bioavailable. The authors therefore tested vacquinol-1 in a xenograft model in which human glioblastoma cells were injected intracranially into mice. Oral administration of vacquinol-1 once daily for 5 days starting 6 weeks after tumour initiation — once large, highly vascularized tumours had developed — rapidly and substantially reduced tumour volume. Furthermore, only two of the eight vacquinol-1-treated animals died during the 80 days of the experiment, whereas all of the eight vehicle-treated animals died by day 60, with a median survival of 31.5 days.

Consistent with their observations in cell culture, the late endosomal/lysosomal marker lysosome-associated membrane glycoprotein 1 (LAMP1) was found in the tumour cells of vacquinol-1-treated animals, but not in those treated with vehicle, suggesting that these cells undergo vacuolization.

Although many of the mutations that occur in glioblastoma are known, attempts to target these mutations directly have not led to therapeutic agents that substantially benefit patients with this disease. Molecules such as vacquinol-1 that are found through phenotypic screening target cancerous phenotypes caused by multiple genetic abnormalities, and could therefore be a good starting point to develop glioblastoma-specific therapeutic agents.

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ORIGINAL RESEARCH PAPER Kitambi, S. S. *et al.* Vulnerability of glioblastoma cells to catastrophic vacuolization and death induced by a small molecule. *Cell* **157**, 313–328 (2014)