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TUMOR RESISTANCE EXPLAINED BY HORMESIS

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□ Enhanced drug (GDC 0449) resistance in a mouse model for human medulloblastoma is shown in the present paper to act via an hormetic response. This has significant implications, imposing constraints on the quantitative features of the dose response of the chemotherapeutic agent, affecting optimal study design, mechanism assessment strategy, potential for tumor rebound, patient relapse and disease outcome.

Keywords: hormesis, biphasic, GDC 0449, medulloblastoma, tumor relapse, hedgehog pathway

Yauch *et al.* (2009) provide evidence of a missense mutation that confers drug resistance to medulloblastoma in a human patient who exhibited a relapse following initially successful chemotherapy. The patient originally was identified with a mutation encoding the inhibitory receptor Patched 1 (PTCH1-W844C), that resulted in up-regulation of hedgehog (Hh) pathway target genes. The patient was orally treated with the Hh antagonist GDC-0449. PET scans three months after initiation of treatment revealed the disease had progressed. The new neoplastic growth displayed markedly enhanced resistance to GDC-0449. The enhanced resistance was not associated with SMO locus amplification but with a heterozygous G-to-C missense mutation in codon 473 with His replacing Asp.

This mutation was subsequently assessed in an *in vivo* murine model. It was referred to as the "corresponding residue" in the human since it is a mutation in the gene forming the SMO receptor of the Hh pathway, involving a change in codon from Asp to Gly, similar to that observed in the patient. The new mouse mutation was associated with a 100-fold increase in resistance to GDC-0449 based on GLI luciferase activity in C3H10T1/2 cells transfected with SMO-WT and SMO-D473G cells derived from allografts from Ptch1+/-, p53-/- mice made resistant to GDC-0449. At GDC-0449 concentrations below the threshold of toxicity in both the parent (SMO-WT) and mutated cells (SMO-D473G) (figure 3C-page 574), a stimulatory response of GLI luciferase activity occurred in both cell types, an observation not discussed in Yauch *et al.* (2009). The

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maximum stimulatory response was 130-135% in the newly mutated cells and 115-118% in the parent cells relative to controls (i.e. 100%). The stimulatory concentration range exceeded 1000-fold in the newly mutated cells but only about 10-fold in the parental cells. These responses indicate a biphasic hormetic concentration-response for both cell types, but with a greater magnitude of stimulatory response in the newly mutated cells based on maximum height and width of the stimulatory response and area under the curve.

In contrast to the responses seen in the mouse model experiments, the GDC-0449 had no treatment effect in the newly mutated human cells. In the parent cells (i.e. initial tumor cells), toxicity was concentration dependent, starting at the lowest concentration (Figure 2B, Yauch *et al.*, 2009). Thus, human cells displayed no evidence of hormesis. Since the newly mutated human cells were not susceptible to the drug while the parent cells were susceptible at the lowest dose, an evaluation of the hormesis hypothesis in the human cells was not possible.

These findings indicate that the response of the mouse cells to GDC-0449 had some similarity with the human cells (i.e. newly mutated cells were more resistant in both the human and mouse and were associated with a similar codon alteration) but nonetheless showed marked quantitative differences, indicating that susceptibility is more complex than can be explained by the shared corresponding codon residue for the SMO receptor.

Despite the interspecies variation in responsiveness to GDC-0449 the observation of hormesis (Calabrese and Baldwin 2001, 2003) is worthy of further consideration. Hormesis is commonly reported in the pharmacological and toxicological literature (Calabrese and Blain 2005), being independent of biological model, endpoint, and chemical agent. Hormesis, a highly conserved strategy by which biological systems adapt to low level exposures to toxic agents, is a dose response characterized by a low dose stimulation and high dose inhibition with consistent quantitative features concerning the magnitude and width of the stimulation. The hormetic response is therefore highly generalizable and extensively reported within animal and human tumor cell lines (Calabrese 2005; Calabrese *et al.* 2006, 2008) including a broad spectrum of brain tumors (Calabrese 2005). Despite widespread occurrence, hormetic dose responses are frequently overlooked, most likely because the low-dose stimulation is modest [30-60% greater than controls; (Calabrese 2008a)], and similar to the Yauch *et al.* (2009) study, investigators focus on responses at higher concentrations (e.g. IC_{50} of therapeutic agents). Since hormetic dose responses are widespread there are a plethora of mechanisms accounting for specific dose responses across a broad spectrum of endpoints, including studies with human tumor cell lines. Despite the diversity of such proximate mechanisms, the quantitative features of the

hormetic dose responses are similar. This suggests the hormetic dose response may represent a quantitative estimate of biological plasticity that is highly generalized.

We suggest the observed increase in resistance as reported by Yauch *et al.* (2009) in their mouse model is a manifestation of hormesis. The enhanced resistance of the mutated mouse cells is observed across the entire concentration range where there is less inhibition at the higher concentrations, and larger stimulation at the lower concentrations relative to controls.

By placing constraints on the quantitative features of the biphasic concentration response, the hormesis model is especially useful for assessing how tumor growth may be stimulated, how the stimulatory response can be switched to an inhibitory mode, how the stimulatory response can be constrained, as well as how tumors may rapidly rebound, accelerating the rate of neoplastic growth and possible relapse. Furthermore, the hormetic dose response may be useful in illustrating the limits within which the rebound/relapse effect occurs, significantly affecting subsequent clinical strategies and disease outcome. While this technical response is focused on the important mutational and experimental findings of Yanch *et al.* (2009), it should be recognized that the hormetic dose response is also a central feature in numerous other biomedical endpoints, affecting memory, bone strengthening, wound healing, hair growth, anxiety, seizure responses, neuroprotection, longevity and numerous other endpoints critical to patient care and the public health (Calabrese 2008b).

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