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Medical Hypotheses (2007) **69**, 537–540

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medical hypotheses

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Why G3139 works poorly in cancer trials but might work well against HIV

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Received 25 December 2006; accepted 7 January 2007

Summary The antisense drug G3139 (oblimersen sodium, Genta, Inc.) is a phosphorothioate oligodeoxynucleotide (ODN) containing unmethylated CpG units, which is targeted to suppress Bcl-2. To date, its effectiveness in cancer clinical trials has been minimal. Some suggestions are provided for that disappointment and recent citations are provided that support the idea that G3139 may be effective at clearing viral infections, specifically HIV. At the time G3139 was conceived as an anti-cancer drug candidate, it was viewed optimistically because Bcl-2 was widely believed to be the most important protein blocking p53-dependent apoptosis caused by internal stress. Since that time, we have learnt that Bcl-2 is not the only protein that inhibits apoptosis and that p53 itself is frequently malfunctioning in tumors. Thus, the anti-cancer utility of suppressing Bcl-2 in cancer cells is limited. Moreover, Bcl-2 has a role in halting the cell cycle (though p27), which may slow down tumor growth; and Bcl-2 even has pro-apoptotic roles in the execution of apoptosis initiated by external death signals (via Fas/CD95 and caspase 3). Overall, in the clinical setting, G3139 usually has statistically significant but medically unimportant benefit. These results have greatly diminished the enthusiasm for the drug especially when the side effects are considered. Specifically, the unmethylated CpG ODN (and/or the phosphorothioate group) activates the immune system, but this potentially important anti-cancer effect is lost when the immune cells undergo premature apoptosis apparently because their Bcl-2 levels have been lowered by the antisense effect of G3139. While this effect on immune cells is usually undesirable, it is exactly what would be useful for activating immune cells, initiating provirus transcription in retrovirus-infected cells, and facilitating selective apoptosis of these infected cells. In general, G3139 might have benefit in clearing chronic infections by intracellular parasites including viruses (HIV, SIV, HTLV, HBV, coronavirus, etc.). Indeed, G3139 has been shown to cause apoptosis in EBV-infected cells leading to clearance of the virus.

The antisense drug G3139 (oblimersen sodium, Genta, Inc.) is a phosphorothioate oligodeoxynucleotide (ODN) containing unmethylated CpG units, which is targeted to suppress Bcl-2 [1–4]. It was expected that reduction of Bcl-2 levels would facilitate apoptosis in drug-resistant cancer cells [1,2]. Over the last several years [3,4], G3139 has been evaluated in several phase III cancer clinical trials and (unfortunately) it only slightly improves the performance of classical chemotherapeutic agents. I hypothesize that this disappointment is in part due to the fact that most cancer clones have lost p53 activity [5]. Internal stresses of various sorts (metabolic, physical, genotoxic) are communicated to p53 via independent signaling pathways

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0306-9877/$ - see front matter
doi:10.1016/j.mehy.2007.01.044

and p53 integrates them into a single level of cellular stress that activates p21 to slow down the cell cycle [6]. If delaying the cell cycle does not allow the cellular stress to be mitigated (e.g., by DNA repair; restoration of pH, Eh or nutrient levels; metabolism or excretion of toxins), then activated p53 reaches a level that summons pro-apoptotic proteins (e.g., Bax and Bak), which initiate apoptosis [7]. Bcl-2 fine-tunes the threshold of stress at which apoptosis occurs by blocking the action of the pro-apoptotic proteins (Fig. 1).

This p53-dependent pathway is apparently absent in the majority of cancer clones as shown by the utility of p53 gene therapy (e.g., with Ad5CMV-p53, Advexin(R), Introgen Therapeutics, Inc.) [8]. Thus, suppression of Bcl-2 in combination with classical chemotherapy, which induces internal stress (e.g., genotoxicity), cannot have much positive effect on apoptosis of abnormal cells [4]. On the other hand, it would appear that a combination of drugs that: (i) restores p53 activity, (ii) suppresses Bcl-2, (iii) induces internal stress in cancer cells, and (iv) suppresses cell fusion [9] would be an attractive candidate for clinical trials.

Another possible reason that G3139 has been of little benefit in cancer clinical trials has to do with the fact that Bcl-2 works to suspend the cell cycle via p27 [10–12]. In some cancers, this may be the only inhibition remaining to block an uncontrolled cell cycle. Obviously, in this case, suppressing Bcl-2 would be undesirable unless it led immediately to apoptosis.

Finally, G3139 has another important feature. Namely, it is an unmethylated CpG compound that has been extensively tested in clinical trials. Unmethylated CpG compounds are known to stimulate immune response by activating leucocytes [13]. This would appear to have desirable anti-cancer effects and may, indeed, account for some of the G3139 benefit in pre-clinical studies, but as shown in Fig. 1, Bcl-2 actually plays an important role in inducing apoptosis triggered by external death signals. Specifically, binding of Fas (CD95) on the cell surface (by FasL, TNF, etc.) activates caspase-3, which cleaves Bcl-2 into pro-apoptotic Bax-like fragments [14]. Ironically, G3139 suppresses Bcl-2 such that the benefit of the enhanced immune response (external stress) against tumors appears to be largely lost.

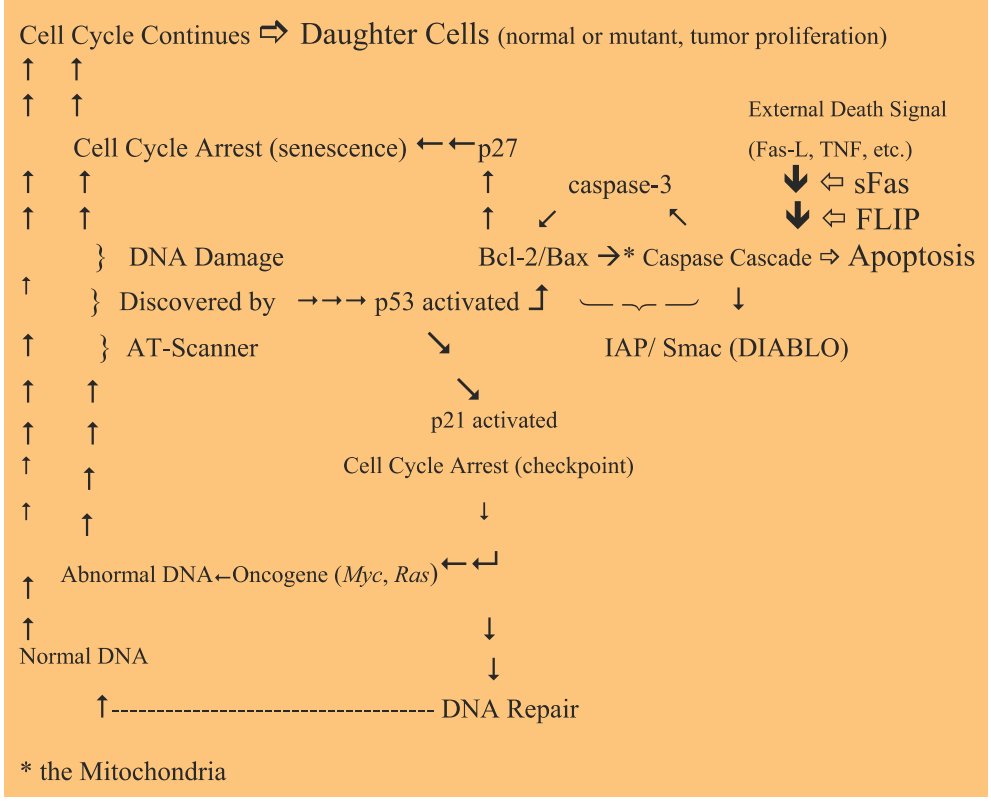

Figure 1 Summary of major pathways leading to apoptosis following damage to DNA that can lead to mutations.

Indeed, this effect of G3139 appears to lead to significant adverse toxicity in cancer trials. G3139 has been shown to suppress Bcl-2 in T-cells, B-cells and monocytes [3]. The combination of activation of immune cells and blocking their Bcl-2 expression leads to substantial apoptosis and neutropenia and thrombocytopenia [4]. It seems clear that G3139 is having an effect in the cells that become infected with HIV [15,16]. It is probably undesirable to treat individuals who have very low levels of T-cells with G3139. If, however, T-cell levels have been stabilized with HAART [17], then G3139 may well activate transcription of the latent HIV provirus inducing *internal* stress in the infected T-cells that is not present in the uninfected (bystander) T-cells. Bcl-2 over-expression is essential for the survival of HIV-infected cells that are actively transcribing the provirus [18,19]. Hence, without the benefit of elevated Bcl-2, these activated, HIV-infected T-cells should preferentially undergo apoptosis. At the same time, lowering the Bcl-2 levels in bystander T-cells makes them less susceptible to *external* death signals. Thus, suppression of Bcl-2 should concurrently protect uninfected cells from the ravages of HIV-infected T-cells.

This hypothesis was originally published in 2004 [20,21] and to my knowledge, no one with means to test it has given it serious consideration. The results of G3139 clinical trials have greatly reduced the enthusiasm for G3139 as an anti-cancer drug. But, the report [22] that G3139 has successfully eliminated EBV+ nasopharyngeal carcinoma xenografts in SCID mice suggests that the drug does eliminate the barriers to viral (internal) stress induced apoptosis. If it turns out that HIV suppresses cellular p53 [23,24] as well as causing over-expression of cellular Bcl-2, then steps to restore p53 function might be necessary.

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