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## Correspondence

# In response to "Selective serotonin reuptake inhibitors—A new modality for the treatment of lymphoma/leukaemia?" by C. Schuster, et al. [Biochem. Pharmacol. 74 (9) 2007 1424–1435]

*Dear Sirs,*

We read with great interest the above paper from Schuster and colleagues. The study is well performed and we believe has merit. As the take-home message of the authors is aimed squarely against our recent and ongoing work we felt it important to respond by contesting their assertion that: "SSRIs are unlikely to represent useful lead compounds for inducing apoptosis in B-cell derived tumours". We take the opportunity here to present to the readership of Biochemical Pharmacology, together with the wider scientific and medical community, additional facts and insight such that an informed judgement to the question posed by these authors can be reached.

In essence, our own studies (encapsulated in Refs. [1,2]) have shown the following:

(1) Early passage, 'biopsy-like' Burkitt's lymphoma cells reveal a heightened sensitivity to the pro-apoptotic actions of SSRI antidepressants compared to the constitutive populations of other (non-BL) lymphoid cell lines; also by comparison to normal resting B cells.
(2) Longer-established BL lines and those that have drifted away from the biopsy phenotype show a relative resistance to the actions of SSRI antidepressants.
(3) Expressing a *bcl-2* transgene affords considerable protection to biopsy-like BL cells from SSRI-promoted apoptosis.
(4) The pro-apoptotic outcome can be achieved with the structurally distinct SSRIs: fluoxetine (Prozac®), paroxetine (Seroxat®) and citalopram (Celexa®).
(5) Regards fluoxetine (for which pharmacodynamic and bioavailability data are most readily available) the concentrations at which biopsy-like BL cells are killed *in vitro* may be achieved safely *in vivo*.

From the above and associated data we are considering fluoxetine in a small Phase II clinical trial of Burkitt's lymphoma where patients have relapsed, or are refractory, to the current cytotoxics vincristine/cyclophosphamide/doxorubicin/methotrexate. The need for new treatment modalities for this dreadful disease, particularly in sub-Saharan Africa where BL remains a childhood killer, was highlighted starkly and eloquently in a recent Lancet essay [3]. Our approach – i.e. 'Drug Reprofiling' – allows leapfrogging over both animal models and Phase I trials by virtue of the compound's safety profile being already approved.

The reasons we suggest that the conclusion reached by Schuster and colleagues of dismissing SSRIs for therapeutic consideration in Burkitt's lymphoma at this stage is wrong are as follow:

(I) The authors failed to include in their study any Burkitt's lymphoma line that could be considered close to the malignant cells as they exist *in vivo*. By their very nature established cell lines are models, at best, for probing function and behaviour of the original tumour. We have deliberately concentrated our efforts on studying "early passage, biopsy-like" BL cells which by all measured criteria remain phenotypically faithful to their *in vivo* precursors. Importantly this includes two of BL's hallmark features: extremely high rate proliferation coupled with high rate spontaneous apoptosis—this seemingly paradoxical pattern being a consequence of the (Ig locus) translocated *myc* gene that defines the disease at a stage in ontogeny when Bcl-2 is not normally expressed. This behaviour helps to explain that while BL is one of the fastest growing tumours it enjoys high cure rates, at least in industrialised nations. Long-term BL lines progres-

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sively lose the background apoptosis inherent to the tumour by it being actively selected against during adaptation to *in vitro* culture: early passage BL cells retain this pivotal trait. In Epstein-Barr virus (EBV)-positive BL artefactual loss of this defining attribute can develop within a year due to *in vitro* liberation of the otherwise suppressed transforming EBV-latent gene programme: this is described as a shift from Latency (LAT) I to Latency III. The three BL lines studied by Schuster and colleagues were first established in the 1960s and two (Namalwa and Daudi) harbour EBV. Where we included one of these (Namalwa) in comparative analysis for a growth arrest response to fluoxetine it was noticeably less sensitive than early passage BL lines while isogenic sublcones of the $EBV^{+ve}$ BL line Mutu were appreciably more sensitive in their natural LAT I state compared to *in vitro*-evolved Lat III variants [1].

(II) For reasons already mentioned, our proposed 'lead compound' for potential translation of SSRIs to BL therapy is fluoxetine [1,2]. Surprisingly, Schuster and colleagues omitted fluoxetine from their study. Instead, they focused on paroxetine and citalopram, which does allow us some comparison. As can be noted from our original report [1], against early-passage L3055 BL cells we obtained $IC_{50}$ values (anti-proliferative) for each of these two SSRI lower than those observed against any of the long-established cell lines investigated by Schuster et al.: $6.9 \pm 1.2$ μM with paroxetine; $20.9 \pm 1.8$ μM with citalopram. Interestingly – and perhaps particularly telling – the single most sensitive line in Schuster and colleagues' work is that generated from murine Eμ-myc tumour cells: as with the early passage BL cells we work with, they seemed to study these within months of isolating *ex vivo* as opposed to decades for the BL lines used. The Eμ-myc cells revealed a level of sensitivity ($IC_{50} = 5.5 \pm 1.46$ μM) to paroxetine nigh identical to that found by us against biopsy-like BL cells [1]. Such agreement between the two studies stimulates confidence in the value of the approach we are suggesting.

(III) We feel persuaded that the heightened sensitivity of (biopsy-like) BL cells to SSRIs (and to pro-apoptotic modalities generally) is due in part to their negligible expression of anti-apoptotic Bcl-2 as evidenced by the protection that is afforded once it is ectopically expressed [1,2]. However, among different cell lines of assorted pathologies additional, even alternative, survival pathways will come into play: as we have indicated ourselves in a separate study with regards sensitivity among a diverse panel of B-cell lines to the pro-apoptotic actions of dopaminergic drugs [4]. Also, even among biopsy-like BL cells (including L3055) survival can be contributed by further Bcl-2 family members and unrelated anti-apoptotic genes such as cIAP2 and A20 [5]. It is therefore unsurprising that Schuster and colleagues found no simple correlation between Bcl-2 levels and sensitivity to paroxetine and citalopram among the broad range of lines they studied. Furthermore, while Bcl-2 strongly protects from the pro-apoptotic actions of antidepressants it affords minimal protection to their anti-proliferative outcomes [2].

(IV) With regards therapeutic considerations, apoptosis is undoubtedly the optimal desired outcome. Unfortunately the single measure used by Schuster et al. to gauge "apoptosis" is Annexin V-binding. Though we have used this when requested by reviewers [1] we prefer to avoid this method and certainly would never employ it as a sole indicator of apoptotic death. As exemplified in Ref. [6] phosphatidylserine exposure (which is what AV-binding detects) is far from restricted to apoptotic outcomes and can accompany multiple activation pathways in a diversity of cells. It is therefore difficult to assess *bona fide* apoptosis from the paper of Schuster and colleagues.

Again we would like to emphasise that despite their main conclusion being an inaccurate and misleading extrapolation, there are elements of Schuster and colleagues' study that make a valuable contribution to this new and exciting field. Particularly pertinent is the demonstration that the major common target for which the SSRIs were first developed as antidepressants – i.e. the serotonin transporter, SERT – is unlikely to be responsible for the anti-lymphoma actions being discussed here. Though our initial rationale for pursuing SSRIs as potential BL therapeutics arose from having identified SERT expression in BL cells, subsequent study has provided us circumstantial evidence leading us to concur that SERT is not the target by which SSRIs exert anti-BL activity [1,2].

Whatever the functional target might be, we feel for the reasons discussed above that it is premature to dismiss SSRIs for consideration as potential treatment adjuncts in Burkitt's lymphoma. We have reviewed the available literature regarding concentrations of fluoxetine that can be reached *in vivo* on current dosing and discussed how these translate to what is observed and necessary for BL cell killing *in vitro* [1,2]. At the current maximal on-license dosing of 80 mg/day (for OCD, obsessive compulsive disorder) the concentrations required for BL kill may be achievable. With these fluoxetine concentrations, neither peripheral blood mononuclear cells nor normal purified B cells succumb to apoptosis; as neither do long-established cell lines, *bcl*-2-carrying BL, nor those that have drifted to an *in vitro* LAT III phenotype. The conventional cytotoxics currently used in treatment can by no means be considered "specific"; even a targeted, rationally designed drug such as Rituximab hits all B cells. The observed heightened pro-apoptotic sensitivity of (genuine) Burkitt's lymphoma cells to fluoxetine indicates a rare 'Therapeutic Window of Opportunity' to be tried and tested in individuals that are still dying from this most terrible disease.

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