

Review

Harnessing serotonergic and dopaminergic pathways for lymphoma therapy: Evidence and aspirations

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Abstract

Growing evidence supports the notion that pharmaceutical targeting of the 5-hydroxytryptamine (5-HT) and dopamine (DA) systems offers the potential to treat human immune system disorders. This review describes this emerging area of research, which has the benefit of being supported by a relatively detailed understanding of these monoamine systems within other tissues of the body. Furthermore, the availability of a number of pharmaceutical agents originally developed to manipulate central monoamine function, offer many suitable drug candidates to test their therapeutic potential in the immune pathology arena.

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1. Introduction

The non-Hodgkin lymphoma (NHL) are cancers of the immune system, arising from the neoplastic expansion of lym-

phocytes and with >90% of examples being of B-cell origin. This group of disease shows a marked spectrum with regards to clinical course ranging from an almost benign indolence to extreme aggression. With regards to incidence, the number of new cases diagnosed in 2007 in the US alone was around 63,000, the number of deaths from NHL, 18,000. The incidence of NHL is increasing substantially, although the reasons for this are unclear. If the number of cases continues to increase at current

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rates, non-Hodgkin’s lymphoma will have an incidence similar to that of breast, colon, lung, and skin cancer by the year 2025 (www.lymphoma-net.org). Despite some exciting advances in treatment options, most notably that delivered by CD20-targeted Rituximab/Mabthera [1], there remains a real and pressing need for novel modalities to manage significant subgroups of NHL stubborn to the current regimens available.

The appreciation that immune cells, including lymphocytes, contain and utilise multiple components more commonly associated with the central nervous system (CNS) leads to novel therapeutic opportunities in the pathologies that derive from them: including the NHL. Specifically: medicaments originally developed for CNS indications could (potentially) be ‘repositioned’ to interrupt/stimulate the relevant pathways in lymphoma cells where the drug targets are identified and shared [2]. The philosophy of this approach led us to the term NeuroImmunoPharmacology [3] and here we discuss its application to the NHL as reflected in pathways impacted by the biogenic monoamines, serotonin and dopamine.

2. 5-HT—the serotonergic pathway

The original name for 5-hydroxytryptamine (5-HT), serotonin, denoted the ability of a serum-derived factor to increase the tone of vascular smooth muscle [4]. Subsequent isolation of the molecule led to the current nomenclature based on its indoleamine structure. This monoamine is now known to mediate a plethora of actions via local hormone and neurotransmitter actions throughout the body [5,6]. For instance in the periphery, 5-HT can both contract and relax smooth muscle (vascular and gastrointestinal), contract cardiac muscle and contribute to blood clotting [6]. In the central and enteric nervous system,

5-HT is utilised by neurones to signal both volumic and fast synaptic neurotransmission [5]. The utility of 5-HT likely arises from its ancient evolutionary past where it is exploited by plants and lower animals.

2.1. Synthesis and metabolism of 5-HT

5-HT arises by enzymatic conversion of the essential amino acid, tryptophan, to the immediate precursor, 5-hydroxytryptophan (5-HTP; Fig. 1). The enzyme responsible, tryptophan hydroxylase, has two isoforms (TPH1 and TPH2) that are segregated in mammals to the periphery and central nervous system, respectively [7,8]. The ubiquitous expression of the aromatic-L-amino acid decarboxylase that converts 5-HTP to 5-HT supports the designation of a cell capable of producing 5-HT by the expression of either TPH1 or TPH2; the TPH enzyme activity being responsible for the rate limiting step of 5-HT synthesis, e.g. [9].

5-HT is metabolised primarily by deamination via mitochondrial monoamine oxidase (MAO) to form 5-hydroxyindole acetaldehyde, which in turn is oxidized by the avid enzyme aldehyde dehydrogenase such that the principle metabolite is 5-hydroxyindole acetic acid (5-HIAA). Of the two isoforms of MAO, MAO-A is primarily responsible for 5-HT’s metabolism [6].

2.2. 5-HT receptors and transporter

The multiplicity of the actions of 5-HT arises in part by the relatively high number of cell surface receptors that communicate 5-HT’s interaction. Unique among bioactive amines, the receptors fall in to two superfamily categories; the G-protein-

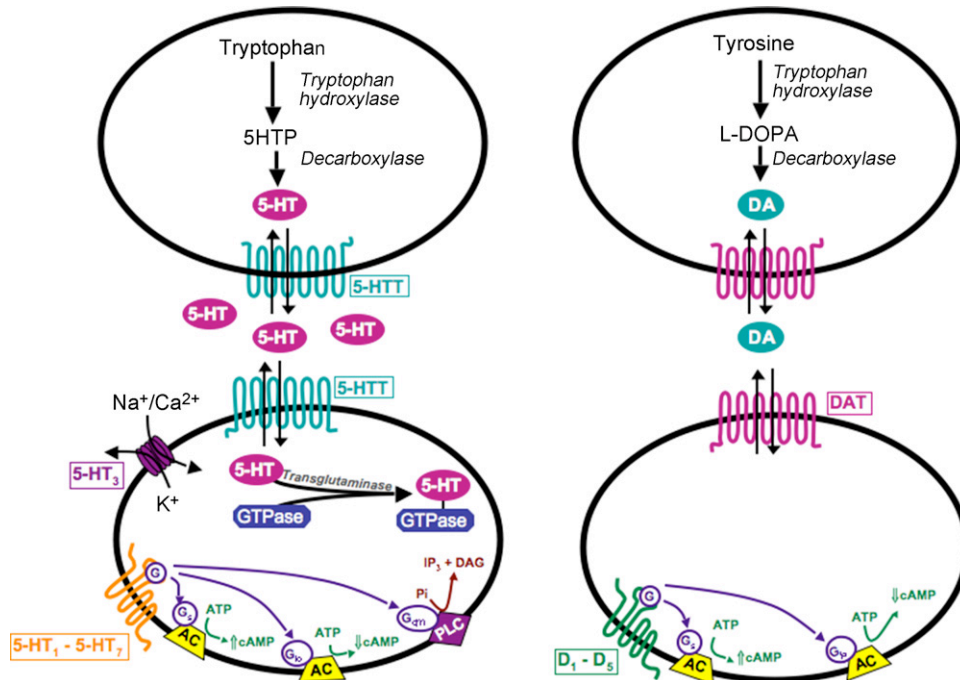


Fig. 1. Schematic representation of cell derived 5-HT and DA and their protein targets with associated transduction systems.

Table 1
Pharmacology of 5-HT receptors and the transporter

Nomenclature	5-HT _{1A}	5-HT _{1B}	5-HT _{1D}	5-HT _{1E}	5-HT _{1F}	5-HT _{2A}	5-HT _{2B}	5-HT _{2C}	5-HT ₃	5-HT ₄	5-HT _{5A}	5-HT _{5B}	5-HT ₆	5-HT ₇	5-HTT
Example selective agonist	8-OHDPAT	Sumatriptan	PNU10929	None	LY334370	DOI	Ro600175	DOI	<i>Meta</i> -CPBG	BIMU8	None	None	None	None	N/A
Example selective antagonist	WAY100635	SB236057	SB714786	None	None	Ketanserin	RS127445	SB242084	Granisetron	RS100235	SB699551	None	SB271046	SB656104	Paroxetine (inhibitor)
Effector coupling	G _{ir/o}	G _{ir/o}	G _{ir/o}	G _{ir/o}	G _{ir/o}	G _{q/11}	G _{q/11}	G _{q/11}	Integral cation channel	G _s	G _{ir/o}	Not identified	G _s	G _s	N/A

coupled seven transmembrane domain receptors (GPCR) and the four transmembrane subunit pentameric cys–cys loop ligand-gated ion channel (LGIC) receptors (Table 1; [5]).

The molecular and functional diversity of 5-HT receptors is further extended by alternative splicing of mRNA, mRNA editing (5-HT_{2C} receptor) and differential subunit incorporation into the receptor complex (5-HT₃ receptor) [5].

Termination of cell surface 5-HT receptor activation is facilitated by the 5-HT transporter, 5-HTT (SERT). This member of the Na⁺/Cl⁻-dependent transporter superfamily displays the characteristic twelve transmembrane domains and consistent with other members, actively transports the substrate down the concentration gradient such that movement can be in either direction [10]. Hence, 5-HTT can not only remove the monoamine from the extracellular environment but under certain conditions can also pump 5-HT out of cells. Furthermore, it is now appreciated that intracellular 5-HT is not simply passive since the ‘serotonylation’ of small GTPases, such as Rac and Rho, directly impacts signal transduction via an initial activation and subsequent degradation of small GTPases [11,12].

3. DA—the dopaminergic pathway

Around the same time as the identification of 5-HT, another monoamine, dopamine (DA), was identified as a functional biochemical and not simply a precursor of the better known catecholamines of the time, noradrenaline and adrenaline [13]. The DA system offers targets to treat a number of neurological and psychiatric conditions (e.g. Parkinson’s disease, schizophrenia) and is also provides the neurochemical basis of reward; including that from drugs of abuse.

3.1. Synthesis and metabolism of DA

DA also arises from an amino acid, tyrosine, via the enzyme tyrosine hydroxylase that forms the immediate precursor, L-DOPA (Fig. 1), in the rate limiting step for DA production. L-DOPA is converted by an aromatic-L-amino acid decarboxylase, to generate DA [14].

DA is metabolised via two potential routes; either a direct action of mitochondrial monoamine oxidase (MAO), the MAO-B isoform, to form dihydroxyphenylacetic acid (DOPAC) or sequentially by catechol-*O*-methyltransferase (COMT) and MAO to form homovanillic acid (HVA).

3.2. DA receptors and transporter

The five human dopamine receptors (D₁–D₅ receptors) are all members of the GPCR superfamily, with the D₂ receptor expressed as two forms termed D_{2S} (short) and D_{2L} (long) arising from alternative splicing of the mRNA [15]. All five DA receptors appear to influence cell biology via a G-protein mediated modulation of adenylate cyclase; typically D₁ and D₅ receptors (the ‘D₁-like’ dopamine receptors) increasing and the D₂, D₃ and D₄ receptors (the ‘D₂-like’ dopamine receptors)

Table 2
Pharmacology of DA receptors and the transporter

Nomenclature	D1	D2	D3	D4	D5	DAT
Example selective agonist	(+) SKF81297	(+) PHNO	PD128907	PD168077	None	N/A
Example selective antagonist	SCH23390	Raclopride	S33084	L745870	None	GBR12909 (inhibitor)
Effector coupling	G _s , G _{olf}	G _{i/o}	G _{i/o}	G _{i/o}	G _s	N/A

decreasing the production of the intracellular second messenger, camp (Table 2).

In addition to extracellular metabolism (COMT), extracellular DA concentration is influenced by the DA transporter (DAT); structurally and functionally similar to SERT consistent with their common membership of the Na⁺/Cl⁻-dependent transporter superfamily [10].

4. Roles in immune system

We have recently reviewed the published data which support an involvement of both the serotonergic and dopaminergic pathways in immune system regulation and behaviour with a focus on lymphocytes [3,16,17]: the targets for oncogenic transformation in NHL. Salient points can be summarised as follows:

4.1. The serotonergic pathway

The potential for immune cell exposure to serotonin comes from a number of routes. Platelet-stored 5-HT can be released rapidly at sites of inflammation by triggers such as platelet activating factor, complement component fragments, and IgE-containing immune complexes [3,16]. Lymphoid organs are innervated with neurons that may be serotonergic in nature under certain circumstances. Both B cells and activated T cells can cross the blood brain barrier indicating that lymphocytes have the potential to be exposed directly to 5-HT flow both in the periphery and the CNS [3,16]. Finally, recent studies indicate that lymphocytes themselves contain the capacity to synthesise 5-HT directly [17,18]: opening up opportunity for both autocrine and paracrine consequences of 5-HT delivery within immune settings.

Emerging data highlight a diverse array of 5-HT receptor subtypes present on different immune cells; evidence for which we reviewed very recently [17]. The exact function of each of the receptor subtypes on lymphocytes is far from understood and the unravelling of their contributions to outcomes such as, for example proliferation, differentiation, survival, and migration is an area of intense current investigation. A particularly exciting example of this activity emerged from a recent report showing that 5-HT produced by activated murine T cells resulted in their autocrine stimulation as a result of ERK1/2 phosphorylation and NF- κ B activation via constitutively expressed 5-HT₇ receptors [18]. It should be noted that for human T cells, the 5-HT_{1B} receptor has been described as contributing towards a proliferation phenotype [19].

In addition to the 14 receptor subtypes, cells additionally have the potential to respond to 5-HT through the bidirec-

tional serotonin transporter (SERT). From both our published and as yet unpublished work we conclude that lymphoid SERT to be biochemically similar to the transporter contained in all (non-platelet) peripheral cells so far studied [20,21]. The major species detected by specific immunoblot resolve to discrete bands of 70 and 60 kDa: the latter available for labelling at the cell surface. Unlike the well-studied SERT generated by over-expression of *sert* cDNA in HEK293 cells which reaches the surface and is expressed abundantly as a heavily *N*-glycosylated 85–95 kDa protein, surface-accessible SERT in lymphoid cells is non-glycosylated and relatively sparse: yet still capable of functional 5-HT uptake [22]. Importantly, as initially described for platelets and more recently for vascular smooth muscle cells SERT can function in cells to promote the ‘serotonylation’ of small GTPases such as Rac and Rho and thereby impact signal transduction directly [11,12]. 5-HT-dependent transamidation can have biphasic consequences: initial activation of the small GTPase followed by its degradation. Given the central role of such molecules in all cell types, including lymphocytes, this could represent a novel paradigm for consideration in immune cell signalling.

4.2. The dopaminergic pathway

As for serotonin, the innervation of lymphoid tissues can also be dopaminergic in nature: a process that may be heightened during psychological stress [3,16]. Such a pathway would offer the provision of dopamine to immune cells in secondary lymphoid tissues upon release of the catecholamine on sympathetic activation. Again, as recently described for 5-HT, a more established recognition for autocrine dopamine production by lymphocytes is documented: dopamine being actively synthesised in the cells from precursor tyrosine via intermediary L-DOPA; tyrosine hydroxylase being the key rate-limiting enzyme in this pathway [23].

McKenna et al. using flow cytometry and subtype-specific antibodies for labelling reported the distribution of dopamine receptors among human peripheral blood leukocytes [24]. They found expression of the D1-like receptor D₅ and all three subtypes of the D2-like receptor family (see Fig. 1 for a schematic of the dopamine receptor family and their activities). In their study, neutrophils and eosinophils showed moderate expression while T cells and monocytes had low expression of dopamine receptors. B cells and NK cells showed the highest and most consistent levels of the different receptors for dopamine.

In an earlier review we detailed data supporting various roles for different dopamine receptors in impacting immune func-

tion [16]. More recently dopamine has been shown to modify the activity of regulatory T cells (T_{reg}) [25]. This it does via a D_1/D_5 receptor-dependent mechanism. In a separate study, D_3 receptors have been implicated in what has been described as “the neurotransmitter-mediated brain regulation of peripheral T lymphocytes”. Here, activated T-blasts cross the blood brain barrier and are exposed to dopaminergic flow. They then return to the circulation and transmit dopamine-dependent information received in the brain, by cytokine release, for example, to other T cells remaining in the periphery [26]. This novel paradigm could extend to other neurotransmitters (e.g. dopamine) and other immune cells (e.g. B lymphocytes) to further such a brain-to-immune system regulatory dialogue: an exciting proposition that commands further investigation. Sarkar et al. recently reported that stimulation through D_4 receptors in human T cells induces T-cell quiescence, the mechanism for this involving the up-regulation of lung Krüppel-like factor-2 expression through the inhibition of ERK1/ERK2 phosphorylation [27]. Finally, Watanabe et al. have described how naive $CD8^+$ T cells selectively express D_3 (in both humans and mice) and their functional studies led them to conclude that dopamine plays a significant role in migration and homing of naive $CD8^+$ T cells via this receptor [28].

Again, we have reviewed the evidence for lymphocytes expressing an active transporter for dopamine [3]. While there are sufficient data to support the conclusion that lymphocytes express and utilise the dopamine transporter (DAT) the molecular characterization of the molecule is less well developed than the related SERT protein. As with SERT, DAT in lymphocytes may similarly provide a conduit for monoamine-dependent modification of small GTPases thereby impacting signalling and its downstream consequences with respect to cell function directly.

5. Harnessing the monoamine pathways for lymphoma therapy

5.1. Evidence so far

5.1.1. The serotonergic pathway

Burkitt’s lymphoma (BL) is a highly malignant NHL characterized by a 100% mitotic index among the constituent cells. The tumour tends to develop extranodally with a preference for the jaw and abdomen. It can double in size within a day and is a serious health problem in the malarial belts of equatorial Africa, North-eastern Brazil, and Papua New Guinea where it is endemic. Limited medical resources in these regions can compromise the survival rates which can be achieved with current aggressive combination chemotherapy. Outside endemic regions, the incidence of BL has increased dramatically in the past two decades due to its association with HIV infection. A continued need for safe, easily deliverable and inexpensive alternatives to bolster the current therapeutic arsenal against BL provided our driver for the application of the NeuroImmunoPharmacology approach to this highly aggressive lymphoma. This culminated in studies identifying the serotonin transporter as an unexpected candidate target for therapeutic attack in the non-Hodgkin’s lymphoma: first in BL then more broadly in NHL in general.

BL cells were shown to carry immunoreactive SERT and to transport 5-HT (serotonin) with appropriate first order kinetics [20]. Moreover, a capacity for 5-HT to drive rapid and extensive apoptosis in biopsy-like BL cells was largely reversed by the Selective Serotonin Reuptake Inhibitors (SSRI) class of antidepressants that block the active uptake of 5-HT into SERT-carrying cells [20]. Subsequently, SERT was shown to be readily detectable in derived B-cell lines with origins as

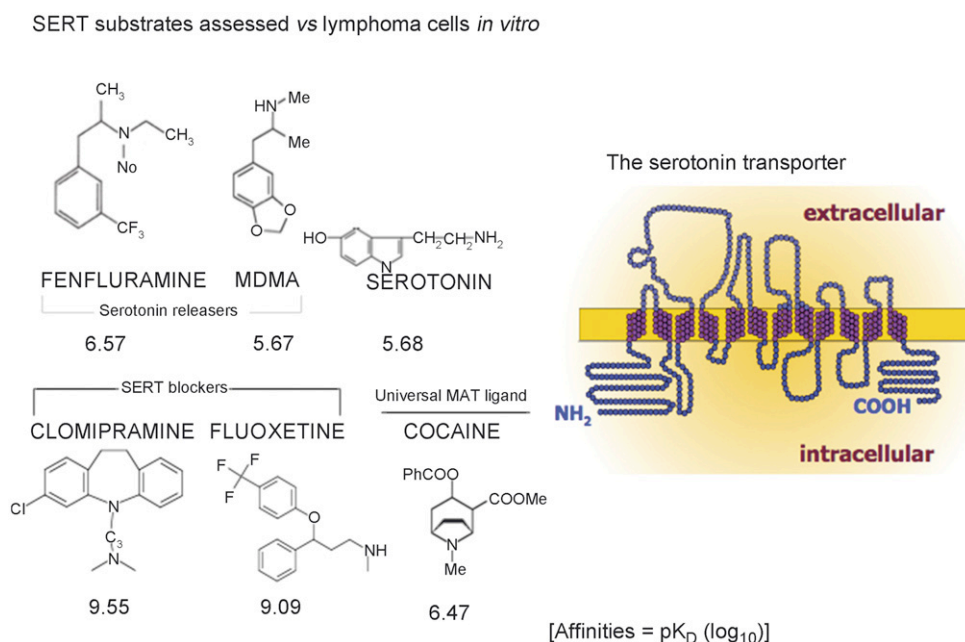


Fig. 2. SERT substrates assessed versus lymphoma cells *in vitro*.

diverse as B-cell precursor acute lymphoblastic leukaemia, mantle cell lymphoma, diffuse large B-cell lymphoma, and multiple myeloma with anti-proliferative actions from 5-HT evident in a proportion of these [21].

While these initial findings indicated a novel strategy in the management of NHL (and Burkitt's lymphoma particularly), serotonin does not of itself offer a viable therapeutic compound as its characteristics are unsuited to that of a deliverable drug. This led us to investigate alternative SERT-binding compounds for potential anti-lymphoma actions [21,29]. The range of SERT substrates assessed by us for their anti-lymphoma is summarised in Fig. 2 together with the affinity they display for transporter binding. These include SSRIs (such as fluoxetine) themselves, the SERT-selective tricyclic antidepressant chlomipramine, the amphetamine analogues fenfluramine and MDMA/Ecstasy, and the 'universal' monoamine transporter (SERT/DAT/NAT) ligand, cocaine.

Against the spectrum of B-cell neoplasia indicated above we noted the following. The concentration and time-course kinetics for the anti-proliferative and pro-apoptotic activities of the amphetamine derivatives were similar to those of 5-HT; chlomipramine, instead, mirrored the behaviour of fluoxetine, both being effective in the low micromolar range. A majority of neoplastic clones were sensitive to one or more of these serotonergic compounds. Dysregulated *bcl-2* expression, either by t(14;18)(q32;q21) translocation or its introduction as a constitutively active transgene, provided protection from pro-apoptotic but not anti-proliferative outcomes [21,29].

The data *in toto* have led us to indicate the potential for SERT as a novel anti-lymphoma target for amphetamine analogues while we believe that the seemingly more promising antidepressants are likely impacting malignant B cells independently of the transporter itself [21]. We are currently actively seeking the non-SERT targets responsible for fluoxetine's anti-tumour actions against lymphoma populations generally; and on Burkitt's lymphoma cells specifically.

5.1.2. The dopaminergic pathway

Given the observations already in place regards potentially suppressive actions of dopamine against immune cell function, including their proliferation, we undertook a systematic investigation of anti-lymphoma activity from this catecholamine and related compounds. This study has been fully reported [30] and the salient points are as follows:

1. Proliferating normal lymphocytes and dividing malignant clones rapidly arrested on exposure to dopamine in the low (<10 M) micromolar range.
2. The antiparkinsonian drugs L-DOPA and apomorphine were similarly anti-proliferative.
3. With the exception of D4, dopamine receptors D1–D5 were variably expressed among normal and neoplastic B-cell populations, as was the dopamine transporter. Transcripts for D1 and D2 were frequently found, whereas D3 and D5 revealed restricted expression; DAT was detected in most cases.

4. Despite expression of receptors and transporter, pharmacological analysis disclosed that dopamine targeted cycling B cells independent of these structures.
5. Instead, oxidative stress constituted the primary mechanism: dopamine's actions being mimicked by hydrogen peroxide and reversed by exogenous catalase with evidence for the intracellular redox protein thioredoxin contributing protection.

We also observed that, among proliferating clones, growth arrest was accompanied by cell death in populations deplete in anti-apoptotic Bcl-2. Importantly, resting lymphocytes escaped low micromolar dopamine toxicity. While dysregulated *bcl-2* expression prevented oxidative-induced caspase-dependent apoptosis, it conferred only minor protection against dopamine-promoted cytostasis. We concluded that the selective impact of dopamine on lymphocytes that are in active cycle indicated a novel axis for therapeutic intervention: not only in B-cell neoplasia but perhaps also in lymphoproliferative disturbances generally.

6. Current and future aspirations

An early goal is to establish small-scale phase II clinical trial assessing the use of fluoxetine in drug-refractory/relapsed Burkitt's lymphoma patients. We are encouraged that the concentrations of SSRIs shown to be active against BL cells *in vitro* are comparable to those reported as being achieved in serum with the current therapeutic use of these drugs for depression and anxiety-related disorders. With the example of fluoxetine, the EC₅₀ of effective BL cell kill at day 6 is around 1 M. For comparison, after 30 days of dosing at 40 mg/day, plasma concentrations of fluoxetine, similarly reach 1 M. SSRIs are also highly lipophilic and accumulate in tissues as noted for the high partition of fluoxetine between brain and blood which could also translate to lymphoma tissues. Moreover, SSRI can be administered at much higher levels than those currently prescribed without major side effects allowing the potential for dose escalation if required.

We would also like to suggest that the amphiphilic nature of both TCA and SSRI antidepressants and their concentration in the phospholipid-rich environment of the brain make them particularly exciting prospects for the treatment of CNS lymphoma. The evolution and origin of primary CNS lymphoma remains a mystery but like all non-Hodgkin's lymphoma continues to increase in frequency and is predominantly a B-cell disease. It is highly aggressive with a particularly poor prognosis, especially in AIDS patients. Secondary CNS lymphoma also tends to be high-grade and is disproportionately frequent among Burkitt's patients. Once more, a small phase II trial would seem appropriate among patients failing the current regimens that are available.

With regards to the amphetamine analogues such as MDMA and Fenfluramine (an appetite suppressant formally marketed as Pondimin[®]), despite their efficacy in arresting BL cells *in vitro*, they are unlikely to constitute a suitable therapy as they stand. First, their anti-lymphoma actions are short-lived: by day

6 of culture with these drugs, the effects on BL population kinetics were little greater than observed at day 1. Second, concentrations of the amphetamine derivatives needed to affect a response are appreciably above those associated with their *in vivo* safety. While we accept that the concentrations of MDMA and FEN required to elicit an anti-lymphoma effect are high, their efficacy nonetheless indicates a potential for amphetamine analogues in this therapeutic context. We suggested that perhaps by redesigning these “designer drugs” we could achieve increases in desired anti-lymphoma activity with concomitant reductions in unwanted neurotoxicity [21]. With active collaboration from Matthew Piggott and his colleagues at the University of Western Australia such a study is now underway.

As already discussed for 5-HT earlier, dopamine of itself is not a deliverable therapeutic. We did find however that apomorphine, an antiparkinsonian drug also used to treat erectile dysfunction, was as effective as dopamine with regards to killing BL cells *in vitro*. Nevertheless, concentrations required to achieve successful killing *in vitro* is 100 times that reached on current dosing *in vivo*. There is evidence that lymphocytes from Parkinson’s disease (PD) patients receiving high-dose 1-DOPA have increased dopamine content such that encouraging its release might generate a sufficient local source to drive lymphoma cell apoptosis though this is currently speculative only. Of more immediate promise are ongoing developments in relevant drug-delivery systems: e.g. by combining the liposomal packaging of 1-DOPA for treating PD with the principle of B-cell targeted ‘immunoliposomes’ for lymphoma [30].

In conclusion, the identification of active serotonergic and dopaminergic component expression in the constitutive cells of non-Hodgkin’s lymphoma populations indicates novel targets and routes of therapeutic attack. The panoply of tried-and-tested and well-tolerated drugs already available for other (currently non-cancer) indications that target these systems allows the possibility of rapid translation to the lymphoma clinic obviating the need for both animal testing and phase I safety trials. Thus, reprofiling/repositioning (‘reinvention’ [2]) and/or redesigning of existing serotonergic and dopaminergic drugs represent a new approach to tackle a continued therapeutic need in the management of (at least subgroups of) the B-cell malignancies.

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References

[1] Mihelic R, Kaufman J, Lonial S, Flowers C. Maintenance therapy in lymphoma. *Clin Lymphoma Myeloma* 2007;7:507–13.
 [2] Editorial. Reinvention. *Lancet Oncol* 2006;7:785.
 [3] Gordon J, Barnes NM. Lymphocytes transport serotonin and dopamine: agony or ecstasy? *Trends Immunol* 2003;24:438–43.
 [4] Rapport M, Green AA, Page IH. Serum vasoconstrictor (serotonin). IV. Isolation and characterisation. *J Biol Chem* 1948;176:1243–51.
 [5] Barnes NM, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology* 1999;38:1083–152.

[6] Fozard JR. The peripheral actions of 5-hydroxytryptamine. Oxford University Press; 1989.
 [7] Walther DJ, Bader M. A unique central tryptophan hydroxylase isoform. *Biochem Pharmacol* 2003;66:1673–80.
 [8] Ledley FD, Grenett HE, Bartos DP, van Tuinen P, Ledbetter DH, Woo SL. Assignment of human tryptophan hydroxylase locus to chromosome 1: gene duplication and translocation in evolution of aromatic amino acid hydroxylases. *Somat Cell Mol Genet* 1987;13:575–80.
 [9] Grahame-Smith DG. Tryptophan hydroxylation in brain. *Biochem Biophys Res Commun* 1964;16:586–92.
 [10] Masson J, Sagné C, Hamon M, El Mestikawy S. Neurotransmitter transporters in the central nervous system. *Pharmacol Rev* 1999;51:439–64.
 [11] Walther DJ, Peter JU, Winter S, Höltje M, Paulmann N, Grohmann M, et al. Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. *Cell* 2003;115:851–62.
 [12] Guilluy C, Rolli-Derkinden M, Tharaux PL, Melino G, Pacaud P, Loirand G. Transglutaminase-dependent RhoA activation and depletion by serotonin in vascular smooth muscle cells. *J Biol Chem* 2007;282:2918–28.
 [13] Iversen SD, Iversen LL. Dopamine: 50 years in perspective. *Trends Neurosci* 2007;30:188–93.
 [14] Fitzpatrick PF. Tetrahydropterin-dependent amino acid hydroxylases. *Ann Rev Biochem* 1999;68:355–81.
 [15] Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. *Physiol Rev* 1998;78:189–225.
 [16] Meredith EJ, Chamba A, Holder MJ, Barnes NM, Gordon J. Close encounters of the monoamine kind: immune cells betray their nervous disposition. *Immunology* 2005;115:289–95.
 [17] Gordon J, Barnes NM. Serotonin: a real blast for T cells. *Blood* 2007;109:3130–1.
 [18] León-Ponte M, Ahern GP, O’Connell PJ. Serotonin provides an accessory signal to enhance T-cell activation by signaling through the 5-HT7 receptor. *Blood* 2007;109:3139–46.
 [19] Yin J, Albert RH, Tretiakova AP, Jameson BA. 5-HT(1B) receptors play a prominent role in the proliferation of T-lymphocytes. *J Neuroimmunol* 2006;181:68–81.
 [20] Serafeim A, Grafton G, Chamba A, Gregory CD, Blakely RD, Bowery NG, et al. 5-Hydroxytryptamine drives apoptosis in biopsylake Burkitt lymphoma cells: reversal by selective serotonin reuptake inhibitors. *Blood* 2002;99:2545–53.
 [21] Meredith EJ, Holder MJ, Chamba A, Challa A, Drake-Lee A, Bunce CM, et al. The serotonin transporter (SLC6A4) is present in B-cell clones of diverse malignant origin: probing a potential anti-tumor target for psychotropics. *FASEB J* 2005;19:1187–9.
 [22] Chamba A, Holder MJ, Barnes NM, Gordon J. Characterization of the endogenous human peripheral serotonin transporter reveals surface expression without N-glycosylation in lymphocytes. *Biochem J*, submitted for publication.
 [23] Bergquist J, Tarkowski A, Ekman R, Ewing A. Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. *Proc Natl Acad Sci U S A* 1994;91:12912–6.
 [24] McKenna F, McLaughlin PJ, Lewis BJ, Sibbring GC, Cummerson JA, Bowen-Jones D, et al. Dopamine receptor expression on human T- and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. *J Neuroimmunol* 2002;132:34–40.
 [25] Cosentino M, Fietta AM, Ferrari M, Rasini E, Bombelli R, Carcano E, et al. Human CD4+CD25+ regulatory T cells selectively express tyrosine hydroxylase and contain endogenous catecholamines subserving an autocrine/paracrine inhibitory functional loop. *Blood* 2007;109:632–42.
 [26] Ilani T, Strous RD, Fuchs S. Dopaminergic regulation of immune cells via D3 dopamine receptor: a pathway mediated by activated T cells. *FASEB J* 2004;18:1600–2.
 [27] Sarkar C, Das S, Chakroborty D, Chowdhury UR, Basu B, Dasgupta PS, et al. Cutting edge: stimulation of dopamine D4 receptors induce T cell quiescence by up-regulating Krüppel-like factor-2 expression through inhibition of ERK1/ERK2 phosphorylation. *J Immunol* 2006;177:7525–9.

- [28] Watanabe Y, Nakayama T, Nagakubo D, Hieshima K, Jin Z, Katou F, et al. Dopamine selectively induces migration and homing of naive CD8+ T cells via dopamine receptor D3. *J Immunol* 2006;176:848–56.
- [29] Serafeim A, Holder MJ, Grafton G, Chamba A, Drayson MT, Luong QT, et al. Selective serotonin reuptake inhibitors directly signal for apoptosis in biopsy-like Burkitt lymphoma cells. *Blood* 2003;101:3212–9.
- [30] Meredith EJ, Holder MJ, Rosén A, Lee AD, Dyer MJ, Barnes NM, et al. Dopamine targets cycling B cells independent of receptors/transporter for oxidative attack: implications for non-Hodgkin's lymphoma. *Proc Natl Acad Sci U S A* 2006;103:13485–90.