Journal of Bioanalysis & Biomedicine

Loudon, J Bioanal Biomed 2013, 5:4
DOI: 10.4172/1948-593X.1000086

Research Article Open Access

Repurposing Amlexanox as a 'Run the Red Light Cure-All' with Read-through – a 'No-Nonsense' Approach to Personalised Medicine

John A Loudon*

Wetherill Park Medical Centre, Suite 101, Stockland Mall, Polding Street, Australia

Abstract

The burden of inherited diseases in terms of congenital defects and cancer predisposition in addition to a multitude of neurological, cardiovascular and other organ diseases arising sporadically during life is enormous on the individual, immediate family and society globally. I approach this major burden to human society by invoking the powerful technology behind read-through, whereby disease-causing substitution nonsense mutations, oftentimes at the root of human diseases, are rescued. Further, the importance of nonsense mutations in the realm of developmental factors linked to cancer stem cell maintenance is presented. The methodology behind the technique is given and proof of concept behind its use *ex vivo* and in clinical trials. The discovery of a novel read-through drug, Amlexanox, which has been in use for over twenty years in dentistry for oral ulcerations, represents a next generation read-through agent. This agent has been demonstrated in cell lines to correct functional loss in cystic fibrosis CFTR, dystrophin and the tumour suppressor, p53. Its novel ability to inhibit nonsense mediated decay is discussed. Amlexanox is therefore presently ready for testing in a wide variety of *in vivo* models of human diseases and also in clinical trials. Given the safety profile of Amlexanox and *ex vivo* efficacy in studies thus far it is envisaged that this accepted medication shall successfully debut as an *all-purpose* agent for the prevention and management of human diseases.

Keywords: Amlexanox; Aminoglycoside; Ageing; Cancer stem cell; Cardiovascular disease; Congenital defect; Cystic fibrosis; Cerebral palsy; Duchenne muscular dystrophy; Epilepsy; Inherited disease; Nonsense mutation; Neurological disease; Read-through; Thalassaemia

Introduction

Importance of nonsense mutations in human disease

My first discussion of the value of read-through therapy for human genetic disease was presented with a focus on cancer [1]. The aim was to rescue substitution nonsense mutations – premature termination codon (PTC) – *via* read-through. My intention was to present a valuable addition to the armamentarium of drug treatments for cancer that would be patient compliant, safe, as well as effective. Since then I have outlined the use of read-through for a variety of human diseases [2-4] including heart and lung and a range of haematologic malignancies. The following presents an over-view of the impact nonsense mutations have in regard human diseases and processes. The list is not exhaustive and further discussion of relevant instances underscoring the importance of PTCs is given in later Sections.

Nonsense mutations result in ~20% of transmitted or *de novo* germline mutations [5-7]. Also these mutations lead to a high percentage of germline and somatic genetic defects resulting in cancer [1,8,9]. There are ~7000 transmitted disorders in humans and 10s of millions of people Worldwide are afflicted with genetic diseases [10]. As ~11% of all human mutations are nonsense mutations [11] many millions could be candidates for targeted PTC rescue therapeutics. Thus examples of PTC percentage of burden are readily found: *beta*-thalassaemia - 70% [6]; cystic fibrosis - >8% [12]; Rett syndrome – 35% [13]; Duchenne muscular dystrophy (DMD) - ~13% [14] and Pseudoxanthomaelasticum- ~35% [15]. In Ataxia-Telangiectasia (AT) patients ~14% of ATM kinase mutations are PTCs and are related to neurodegenerative disease which in addition carries a heavy cancer predisposition [16]. ATM kinase is part of the DNA damage-response *'machinery'* and is involved in suppressing viral-mediated cellular transformation [17]. In cancer, PTCs often occur in tumour suppressors (TS) [1,11]. Notably, ~10-30% of patients with an inherited cancer predisposition carry nonsense mutations in TS [9]. For example these include such conditions as: familial adenomatous polyposis – ~30% PTCs in the APC gene; Cowden syndrome – 33% PTEN gene PTCs; Li-fraumeni syndrome - ~7% PTCs in p53 and familial mammary gland/ovarian cancer -~18% BRCA1/2 gene PTCs. Also, sporadic TS PTCs [8] occur relatively frequently in cancers (Table 1). Very importantly, PTCs are highly represented in metastatic cancer over primary [18], *viz:* liver metastases: primary colonic cancer present at the proportion of 52%:13%. In regards APC, not only this is a frequent TS mutated in colonic carcinoma it is also sporadically is involved in other cancers such as hepatic carcinoma [19]. That study demonstrated a case of APC nonsense mutation in one allele with the other allele deleted. This investigation provided the first available evidence that genetic inactivation of the APC gene can play a significant role in the progression of sporadic hepatocellular carcinoma. Thus any technique that rescues APC function in such a context would prove to be no doubt beneficial in managing that particular cancer.

Catalogue of somatic mutations in cancer

Continuing with the cancer theme, even a brief examination of COSMIC - Catalogue of somatic mutations in cancer [1] shows the weight PTC mutations carry in the causation of a whole range of cancers through TS inactivation. As such, COSMIC provides for an excellent

***Corresponding author:** John A Loudon, Ph.D. Cert. Oral Path., FAAOMP, BDS Hons, BSc (Dent) Hons. Syd & Ohio, Wetherill Park Medical Centre, Suite 101, Stockland Mall, Polding Street, Wetherill Park, Sydney, NSW, 2164, Australia, Tel: +61-2-9756-3636; Fax: +61-2-9756-3637; E-mail: jon.uk1515@gmail.com

Received July 25, 2013; **Accepted** August 17, 2013; **Published** August 20, 2013

Citation: Loudon JA (2013) Repurposing Amlexanox as a 'Run the Red Light Cure-All' with Read-through – a 'No-Nonsense' Approach to Personalised Medicine. J Bioanal Biomed 5: 079-096. doi:10.4172/1948-593X.1000086

resource to explore the impact of nonsense mutations in sporadically occurring cancers. A summary of the frequencies of commonly mutated TS in a selection of cancers from various sites is presented (Table 1). As a guide for users, when one enters COSMIC home page [8], a selection choice may be made. To fully explore COSMIC, one manner to conveniently search is by 'Tissue' to view mutations in a broad, unselected range of targets. When this is selected, a menu appears of catalogued human tissues. Selection sites include: adrenal gland, autonomic ganglia, bilary tract, bone and so forth. Selecting on any one of these sites, for example, upper aerodigestivetract defines a selection of sub-tissues - in this instance, mouth, pharynx, tonsil, trachea, sinonasal cavity, middle ear, lymph node from the region, larynx, head and neck generally and NS – not otherwise specified. The number of annotated cases – at the updated version of COSMIC from March/April 2013 – is presented in brackets after each listed sub-tissue. By selecting 'mouth' a further window to the right lists the main cancer histology types. In this instance, the vast majority is carcinomas - 904 catalogued cases. By selecting carcinomas a sub-histology menu bar appears to the right listing squamous cell carcinoma (876 cases) as forming the vast majority. Selecting this sub-histology then enables one to 'go to' the gene histogram outlining the twenty most frequently mutated genes for that particular selection/location. This very conveniently assists in determining which gene(s) may be effectively explored in detail to determine the nature of the underlying mutational event. In the case described, p53 TS mutations form the majority of mutations in that tumour in that location. The frequency of mutational events is given in brackets after the gene for example 46% of cases show p53 mutations and 18% of cases show CDKN2A mutations - another key TS. Of course, it must be borne in mind that the mutations listed may not be mutually exclusive and certain catalogued samples may carry more than one of the indicated 'top twenty' mutated genes, or indeed other relevant mutations not appearing in the 'top twenty'. Cancer is, after all, a 'multi-hit' process.

Pursuing the above example further, on selecting p53, a new window appears that lists the amino acid residues altered through the display of a histogram. The full length of the protein is shown from residues 1-394. Tumour source may be selected to list cultured *in vitro* lines *vs.* direct tumour sample. The majority of cases analysed come from tumour samples. Within each of these categories, one may select for mutation type on the right side menu. By selecting tumour source and mutation type as 'substitution nonsense' a histogram appears showing the distribution of nonsense mutations in p53 in tumour samples from mouth squamous cell carcinoma. To examine in detail the precise molecular alteration in each sample select 'Mutations' on the top 'bar' and a convenient listing appears displaying, in this case, 24 entries. The position and mutation, nucleotide change, and amino acid residue replaced by the PTC are shown.

From the summary in Table 1 certain comments may be made. Key TS such as p53 and CDKN2A and PTEN are oftentimes mutated. In certain cancers such as colon and haematologic malignancies, TS such as APC and tet2 present a high level of mutational events. Certain cancers, dependent on sub-histologies selected, appear to demonstrate a particular preference as to which TS may be involved in nonsense mutational events. For example, lung sub-histology adenocarcinoma shows a high percent of STK11 TS nonsense mutations. On the other hand, STK11 is less represented as a nonsense mutational event upon taking lung cancers as an entire grouping. Interestingly though, other TS such as p53 and CDKN2A remain equally represented in terms of nonsense mutational events. The Notch factor, TS in various contexts 'Cancer stem cell factors and nonsense mutations' is highly represented as a nonsense mutational target in certain cancers such as skin and mouth squamous cell carcinoma. A further discussion of the importance of this particular factor is presented later. Those TS mutated outside of the top five mutated genes in each selected case are not included in Table 1 yet this does not in any way minimize their importance. For example, in terms of hepatocellular carcinoma, APC has only four mutations catalogued in COSMIC for this cancer but three of those are frame shift leading to PTC and one is a substitution nonsense mutation. Therefore all collated APC mutations in this cancer lead to PTCs. This supports the importance of PTCs in the APC gene in that cancer [19]. Therefore Table 1 is a conservative demonstration of the causative influence substitution nonsense mutations play in sporadic cancers.

Further examples of nonsense mutations and human diseases - Neurological system: neurodevelopmental disorders

Neurodevelopmental disabilities cover a very broad range of conditions. For example, intellectual disability (ID) is a clinical sign reflecting a diverse series of neurological defects of development ranging from Autism to Attention Deficit Disorder. ID may appear isolated but commonly presents as a syndrome complex. Methyl-CpG-binding domain 5 gene has been associated with a subset of ID. A *de novo* PTC is associated with a particularly severe ID phenotype [20]. In a case of identical twins with intellectual disability and progressive spastic paraplegia, whole exome sequence analysis (WES) revealed a homozygous PTC, p.R1105X, in the AP4E1 gene [21]. Mutations in PAK3 (p21-activated kinase 3) are associated with x-linked non-syndromic ID where there is only one isolated clinical feature *viz:* cognitive deficit. The mechanism may relate to formation and/or plasticity of synaptic networks [22]. PAK3 is heavily expressed in hippocampus and is linked to Rho-GTPase signaling. This forms a link between the actin cytoskeleton and synaptic network formation. Non-syndromic ID is related to production of abnormal neuronal structures *viz:* dendritic spines. The MRX30 mutation of PAK3 (p.R419X) results in specific functional and anatomical defects in nerve architecture and function. These mimic the aberrant architecture of neurons of subjects with non-syndromic ID. Unsurprisingly, MRX30 mutant was later isolated as one of several presumptively causative mutations in non-syndromic ID [23]. Expression of this mutation in hippocampus neurons drastically alters neuronal dendritic spine morphology. One may then conclude that this mutation is disease-causative and a suited read-through target for rescue of the ID.

In other examples, the UPF3B mutation -p.R430X, associates with ID [24] as does the syndrome complex with SALL1 mutation – p.R1054X [25].

Rett syndrome is a neurodevelopmental defect presenting oftentimes with seizures, palsy and autism features. Most cases are sporadic and have severe impairment. A very significant number of nonsense mutations present in the causative gene: MECP2. Interestingly, read-through for Rett syndrome *ex vivo* and with animal models is successful as a proof of concept [13]; Section: 'Proof of concept – read-through']. Novel agents, NB54 and NB84 were defined from the mouse model of Rett nonsense mediated disease, *viz:* MECP2 (p.R168X) [13]. Such agents are touted to be promising candidates for read-through therapy for Rett syndrome patients as they are more effective than gentamicin. Having said this, safety profile for use in human subjects needs to be defined for these novel compounds as well as precise read-through mechanisms [25].

Tissue	Subtissue	Histology	Subhistology	TS	Total mutations	Total SN	% SN
A)Large Intestine	colon	carcinoma	adenocarcinoma				
				p53	455	28	6%
				APC	618	127	21%
				ATM	174	18	10%
				PTEN	227	19	8%
B)Large Intestine	Brain	glioma	All	p53		17	5%
				CDKN2A	39	3	8%
				PTEN	347	34	10%
C) Lung		carcinoma	All	p53	733	65	9%
				CDKN2A	103	11	11%
				Rb1	128	42	33%
D) Lung		carcinoma	adenocarcinoma	p53	314	36	11%
				Stk11	116	25	22%
				CDKN2A	47	4	9%
E) Skin		malignant melanoma	All	CDKN2A	119	17	14%
				p53	75	9	12%
				PTEN	95	11	12%
F) Skin		carcinoma	basal cell carcinoma	p53	121	9	7%
				PTCH1	215	50	23%
G) Skin		carcinoma	squamous cell carcinoma	p53	121	17	14%
				CDKN2A	26	7	27%
				NOTCH1	18	5	28%
				NOTCH2	20	4	20%
H) Liver		Hepato-cellular carcinoma		p53	343	33	10%
				CDKN2A	31	5	16%
I) Mammary		carcinoma	All	p53	835	49	6%
J) Salivary gland		carcinoma	All	p53	31	2	6%
K) Aerodigestive tract	mouth	squamous cell carcinoma		p53	322	31	10%
				CDKN2A	45	10	22%
				Notch 1	24	5	21%
(L) Pancreas		carcinoma	All	p53	262	24	9%

				CDKN2A	101	12	12%
M) Haematopoietic	Haematopoietic and lymphoid malignancies		AML	Tet2	384	99	26%
			CML	CDKN2A	12	1	8%
				Tet2	7	1	14%
			Myelodysplastic syndrome	Tet2	206	56	27%

Footnote:

In (A), under COSMIC tissue selection large Intestine is selected, thence colon subtissue and carcinoma histology and adenocarcinoma subhistology.

In (B), under COSMIC tissue selection central nervous system is selected, thence brain subtissue and glioma histology and select 'all' for subhistology.

In (C), under COSMIC tissue selection select lung, thence 'all' subtissue, thence under histology carcinoma is selected thence, 'all' for subhistology.

In (D), under COSMIC tissue selection select lung, thence 'all' subtissue thence carcinoma for histology thence under subhistology select adenocarcinoma.

In (E), under COSMIC tissue selection select skin, thence 'all' subtissue sites thence malignant melanoma histology thence 'all' subhistologies.

In (F), under COSMIC tissue selection also select skin, thence 'all' subtissue sites and thence under histology select carcinoma, thence under subhistology select basal cell carcinoma.

In (G), under COSMIC tissue selection also select skin, thence 'all' subtissue sites and thence under histology select carcinoma, thence under subhistology select squamous cell carcinoma.

In (H), under COSMIC tissue selection select liver and under subtissue sites select 'all', thence under histology select carcinoma and thence under subhistology select hepatocellular carcinoma.

In (I), under COSMIC tissue selection select breast thence subtissue select 'all', thence under histology select carcinoma thence under subhistology select 'all'.

In (J), under COSMIC tissue select salivary gland and 'all' as subtissue and thence carcinoma as histology thence 'all' as subhistology.

In (K), under COSMIC tissue select upper aerodigestive tract thence mouth as subtissue, thence carcinoma as histology thence under subhistology select squamous cell carcinoma.

In (L), under COSMIC tissue select pancreas thence subtissue select 'all', thence under histology select carcinoma, thence 'all' subhistologies.

In (M), under COSMIC tissue select haematopoietic and lymphoid tissue thence 'all' under subtissue selection thence haematopoietic neoplasm under histology selection thence under subhistology select acute myeloid leukaemia (AML), chronic myeloid leukaemia (CML) or myelodysplastic syndrome.

Table 1: COSMIC (Catalogue of Somatic Mutations in Cancer) analysis showing the frequency of substitution nonsense (SN) mutations from a range of cancers. The selected TS (Tumour Suppressors) are those presenting with mutations that are within the category of the most frequently altered genes for each indicated cancer. Designations (for example, subtissue and subhistology) are as annotated on COSMIC. Total mutations represent deletions/frameshift/insertions/missense/substitution nonsense *in toto* for the indicated TS gene for the particular cancer/histology type as per COSMIC. The percentage of the total alterations for the indicated TS and histology-type that are SN is presented in the last column. COSMIC data extracted July, 2013.

Mutated TS may play a role in neurodevelopmental conditions such as NF1 and NF2. Neurofibromatosis 1 is one of the most common single gene disorders affecting neurological function. This is a developmental disorder caused by germline mutations in neurofibromin, a regulator of the Ras oncogene signal transduction pathway. This disease relates to neural tumours and learning difficulties including Attention Deficit Hyperactivity Disorder (ADHD) and epilepsy. One half of cases are spontaneous and pre-birth screening may be achieved with counseling. At one in 3500 babies born it remains a significantly prevalent condition. Disease-causing nonsense mutations that disrupt the activity of NF1 have been defined [26,27]. Neurofibronatosis 2 produces the TS Merlin and when disrupted leads to tumours of cranial nerve VIII – the auditory- vestibular apparatus. Also, intracranial tumours may be seen such as meningiomas. Although NF2 is notably less frequent than NF1, truncating mutations *viz:*substitution nonsense and frame shifts are common germline events and result in the most severe disease presentation [28]. Interestingly, single/multiple exon deletions are not infrequent yet associate with a milder NF2 phenotype.

Tuberous sclerosis, a rare multisystem genetic disease presents with benign CNS, kidney, heart, eye, lung and skin tumours. This condition may involve seizures and a high percentage has ID and is causatively related to TS mutations in either TSC1 and TSC2 encoding hamartin and tuberin respectively. In the CNS, cortical hamartia or tubers are found. A case of a nonsense mutation in TSC2 which was passed from father to son with tuberous sclerosis features has been examined [29]. Another study has investigated TSC1 [30] and all mutations were truncating.

Other studies point out the presence of nonsense mutations in syndromes with autism and associated ID features [31]. FOXP2, a forkhead transcription factor, shows the presence of a *de novo* nonsense mutation (p.R525X) in the conserved forkhead DNA-binding domain in a patient with autism. Further [32], SHANK2 synaptic scaffolding gene variants are found specific to Autism disorder and ID, including a *de novo* nonsense mutation. Another investigation, with the use of WES, shows that disruptive *de novo* nonsense mutations in brain-expressed genes are associated with autism [33]. Nonsense mutations disrupting the SCN2A (sodium channel, voltage-gated, type II, alpha subunit) in selected affected patients pinpoint this gene as causatively involved in autism. Sodium channels have a relationship to neurodevelopmental disturbances [34]. A protein truncation mutation within the sodium channel SCN8A gene led to cerebellar atrophy, ataxia, and ID. Dravet syndrome is severe myoclonic epilepsy of infancy with psychomotor impairment. Nonsense mutations are common and possibly aetiologically related to the condition. Unfortunately, the seizures tend to be resistant to most anti-epileptic medications. Mutations of SCN1A, a sodium channel alpha subunit, are related to a particular subtype of the disease and nonsense mutations are significantly represented [35]. Crouch gait, a debilitating movement abnormality, is observed in up to 50% adults with SCN1A mutations [36]. A strong correlation was noted between patients carrying SCN1A nonsense mutations and crouch gait. Clearly a therapy is required for management and/or prophylactic prevention. Along these lines, read-through has already demonstrated 'proof of concept' for nonsense mutation rescue for a sodium channel gene, SCN5A in the context of heart disease [37]. This again, as in the case of Rett syndrome [13], establishes read-through as a tangible means for overcoming nonsense-mediated neurodevelopmental disorders.

Epilepsy

Epilepsy is indeed an ancient affliction noted throughout history since Biblical times. Oftentimes, epilepsy may be seen as part of a broader spectrum of phenotypic alterations such as with ID and autism disorder or in an isolated form. Substitution nonsense mutations have been noted in genes that relate to synapse transmission and neuronal stability such as SynapsinI [38]. SynapsinI (SynI) is a synaptic vesicle (SV) phosphoprotein and has a role in synaptic transmission/plasticity by impacting on important steps of SV trafficking both in excitatory as well as inhibitory synapses. Nonsense mutations in human SYNI have a causative role in epilepsy with autism. For example, a SYNI substitution nonsense mutation is associated with higher network excitability. This could then feed mechanisms leading to epilepsy/autism manifestations. Cyclin-dependent kinase-like 5 gene (CDKL5) mutations may also produce epilepsy with degrees of severity depending on the nature of the mutation involved. Other phenotypic features of such gene mutations include neurodevelopmental problems in general [39]. A *de novo* nonsense mutation of the CDKL5 gene, p.R59X, affecting the catalytic domain was found to be involved in a severe disease presentation. As this appears to be located in a structurally sensitive area of the protein then read-through may not replicate function, or indeed it may result in altered function (Read-through 'mechanics', agents and protein 'structure-function' relationship).

Neurodegenerative disorders - motor defects

Hereditary paraplegias (HP) are genetically and clinically mixed group of neurodegenerative disorders. Mutations of SPG4 and SPG3A genes result in ~50 percent of cases of autosomal dominant HP. Of the 10 novel mutations found in SPG4 and SPG3A genes in the families tested in one study the majority of these new mutations were frameshifts or nonsense-type (80%) resulting in loss of protein [40]. Another study shows a novel SPG4 nonsense mutation segregating in a large Northern European family with spastic paraplegia [41]. Other studies confirm the importance of nonsense mutations in these debilitating developmental conditions [42,43].

Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease – a rapidly progressiveneurodegenerative disease affecting muscles. ALS2, producing the Alsin protein, was shown to be the underlying gene linked to development of amyotrophic lateral sclerosis 2 (ALS2). This represents an autosomal recessive form of juvenile ALS and frame shift mutations that generate PTCs in ALS2 are linked to the development of ALS2 variant [44]. More recently, Optineurin gene mutations- specifically a nonsense mutation, p.Gln165X, has been linked to the development of ALS [45].

Another neuromuscular degenerative condition, spinal muscular atrophy (SMA), generally represents defects in SMN1 gene needed for motor neuron survival of cells in the anterior horn of the spinal cord. When these cells degenerate there is concomitant muscle wasting. SMA is the most common genetic cause of infant death and is transmitted in an autosomal recessive fashion. Nonsense mediated decay (NMD) of SMN1 is considered a significant cause of SMN1 degradation with resultant SMA phenotype [46]. Knockdown of UPF1, a factor involved in NMD improved levels of SMN1 transcripts in patient cell lines. Clearly, a readily available NMD inhibitor would be predicted to be beneficial in a subset of SMA patients [46]; (Enter Amlexanox). A nonsense mutation in vaccinia-related kinase1, a serine/threonine kinase that phosphorylates p53 and CREB and is required for nuclear envelope formation, has been causatively related to SMA [47]. This suggests that additional genes are involved in SMA and, more broadly, in neuronal development and maintenance. Finally, and very encouragingly, read-through has provided beneficial evidence from patient cell lines with SMN PTCs and an *in vivo* mouse SMA model [48]. These data are very encouraging in that a cure is on the horizon in respect to selected nonsense-mediated SMA types for what has been to date no available therapy for.

Neurodegeneration - ageing

Ageing may be modeled as a chronic neurologically-driven disease. Examples include dementia, particularly reflecting premature ageing processes with early onset varieties of dementias. Alzheimer's disease, which is a major common form of dementia with memory loss and mood swings, falls into this category. Generally this is assumed to be a neurodegenerative condition with age, along with Parkinson's disease. Interestingly, an early onset familial Alzheimer's disease may present as a marker for premature ageing [49]. Nonsense mutations in the SORL1 gene encoding a protein involved in control of amyloid beta peptide production are involved not only causation of Alzheimer's but, in particular, early onset type. In frontotemporal lobar degeneration, the most common cause of early onset dementia, charged multivesicular body protein 2B (CHMP2B) is considered related to the causation [50]. A nonsense mutation, p.Gln165X, in a family yielding a C-truncated protein has been observed which, when overexpressed, leads to large, aberrant endosomal structures. It is concluded that C-truncating mutations in CHMP2B underlie the pathogenic mechanism in frontotemporal lobar degeneration by disturbing endosomal function [50]. Mutations presenting in progranulin lead to NMD that also plays an important causative role in fronto-temporal lobe dementia [51]. A homozygous nonsense mutation was found in PINK1, PTEN-induced putative kinase1, p.Q456X, associated with a family displaying features of early onset Parkinson's disease [52]. Clearly, carriers of this condition, who are predisposed to Parkinson's disease as well as affected from homozygotes, would seek to benefit from a nonsense mutation rescue strategy.

Overall, this discussion points the health and integrity of the nervous system relating to longevity and in the prevention of premature ageing [53]. Read-through of disease-ageing related nonsense mutations could well be a step forward in preventing premature ageing phenomena in many individuals worldwide.

Vascular and Heart diseases - Aneurysm

Myosin light chain kinase mutations result in familial aortic dissecting aneuryms [54]. A loss of function nonsense mutation in myosin light chain kinase, p.R1480X has been isolated and is presumed to represent a causative mutation. Marfan syndrome is an autosomal dominant and systemic disorder of connective tissue. Dilatation and dissection of the aorta is a manifestation. A novel *de novo* nonsense mutation in fibrillin-1 is seen in a Marfan disease carrier, *viz*:p. Gln2553X, presenting with cardinal disease features [55].

Coronary artery disease(CAD)

Interestingly, the p.R1141X substitution nonsense mutation of the Abcc6 gene is a strong risk factor for CAD [56]. This is not entirely surprising however, as Pseudoxanthomaelasticum, a multiorgan disease related to causative Abcc6 gene mutations, oftentimes presents with early-onset CAD. Importantly, this is a recessive disease and yet carriers also have a high risk for CAD at a very significant odds ratio of 10:5. Therefore carriers of Abcc6 with loss of function mutations

shall still benefit from CAD therapies and are high risk and would conceivably benefit from read-through technology. Importantly, along these lines, read-through has already been shown to be of value for Abcc6 nonsense mutations [15].

Familial hypercholesterolemia is a significant player in CAD. Interestingly, a triple-nucleotide polymorphism leading to 3bps alteration in the low density lipoprotein receptor is considered pathogenic for familial early onset CAD [57]. This is unusual for a stop codon to be generated from 3bps consecutive alteration and points out that SNP arrays may overlook such important disease-causing mutational events. Read-through could be anticipated to place in an amino acid residue that may be then completely unrelated to the one removed by the triple-mutational event – this in turn may have structural/functional implications for such substitutions.

Ironically, correction of nonsense mutations may not always prove beneficial. For example, it has been noted that reduced activity of adenosine monophosphate deaminase AMPD may increase the production of adenosine, a cardioprotective agent [58]. A common nonsense mutation of AMPD1, converting CAA codon to TAA stop at nucleotide 34 in exon 2 (the C34T polymorphism) has been associated with prolonged survival in heart failure and disease. AMPD1 genotyping can demonstrate these individuals in the population and if at risk for cardiovascular disease then read-through of a nonsense mutation for those persons for any other purpose may have to be balanced against possible risk to the heart.

Hypertension

Arterial hypertension is not infrequently seen as secondary to a biochemical defect. For example, steroid 11beta-hydroxylase deficiency is a common cause of congenital adrenal hyperplasia resulting in hypertension [59]. The causative gene, CYP11B1, was analysed in 15 patientcohorts of North African Arab patients and two mutations were found, one nonsense type, *viz*:p.Q356X in 27% cases. Familial glucocorticoid receptor haploinsufficiecny resulting from NMD leads to adrenal hyperplasia and hypertension with attendant cortisol resistance and hypokalaemia [60]. The phenotype associates with a nonsense mutation, p.R469X, within the second zinc finger of the DNA binding domain of the receptor.

Obesity and diabetes

Metabolic diseases such as diabetes and associated obesity are commonplace. A nonsense mutation, p.R392X, has been designated within the TLR5 gene encoding an innate immunity receptor. This nonsense allele protects from obesity in an Arab population but was found to be a risk factor for type II diabetes. In contrast to the animal model of this mutation loss in humans of this functioning allele protects from weight gain. Thus in the mouse model the animals gain weight but in correspondence with humans the allele is a risk for type II diabetes [61]. These studies confirm immune dysregulation as a central player in metabolic diseases. Read-through may be anticipated to rescue this mutation and correct the dysregulation and type II diabetes in humans. However, it may also predispose to obesity in that context. Interestingly, the next generation read-through agent being presented as the theme in my article has properties that in themselves serve to address diabetes and obesity (Enter Amlexanox) – though the mechanism in that instance is not *via* read-through. It is interesting to speculate that this may counteract read-through rescue of obesity mechanisms operated by functional TLR5.

Oral pathologies

Periodontal diseases, affecting the supporting structures of the teeth, are very prevalent around the world. In certain presentations of this disease an inherent susceptibility may be seen, for example in Papillon–Lefèvre syndrome. This condition is autosomal recessive and represents a severe aggressive periodontitis resulting from mutations in cathepsin C gene. Truncation mutations are considered pathogenic [62]. For example, a homozygous cathepsin C mutation resulting in a substitution nonsense mutation of cysteine to stop at position 30 in exon 1 was considered causative in a Papillon–Lefèvrepatient who had carrier parents [63].

By WES technique, a nonsense FAM20A mutation is implied as causative for gingival hyperplasia syndrome with amelogensis imperfect – a disfiguring condition of the teeth [64]. The gingival hyperplasia has implications for periodontal health. Many other conditions relate to dental health. For example, RUNX2 nonsense mutations leading to cleidocranialdysostosis have significant dental features [65].

Intermsofcancers,ithasbeennotedthatgermlinePTCsarecausatively involved in aberrant telomere biology in dyskeratosiscongenita. This condition oftentimes presents with mouth pre-cancers, called leukoplakia [66]. In basal cell carcinoma (BCC) PTCH mutations are not uncommonly seen Table 1 and are TS mutations. Defects of PTCH have been implied to be associated with syndromes associated with BCC as well as with non-syndromicodontogenickeratocysts [67]. Nevoid basal cell carcinoma syndrome (NBCCS) is autosomal dominant and presents with BCC and keratocysts. There are multiple organ defects to be noted as well. The odontogenickeratocysts of the jaws present in adolescence. The responsible gene is PTCH1. In one case, the SUFU gene p.Q184X nonsense mutation was observed. SUFU is downstream of PTCH1 in the sonic hedgehog signaling pathway [68] and the SUFU germline mutation in this case was presumably causative of NBCCS. Further, SUFU gene mutations carry a high risk for medulloblastoma and meningioma in addition to the typical NBCCS presentation. Interestingly too, in adenoid cystic carcinoma, another oral pathology but of a particularly malignant nature involving the salivary glands, somatic truncating mutations were seen in several genes including SUFU [69].

Lesions in other TS genes also relate to mouth cancers (Table 1).

In summary, the major impact nonsense mutations make in terms of human disease from dental pathologies to neurological conditions and cancer and heart disease can be readily appreciated from this discussion (Figure 1).

Read-through 'mechanics', agents and protein 'structure-function' relationship

Nonsense mutations produce truncated proteins that are non-functional and/or unstable and may result in dominant negative effects. Further, the associated mRNA is unstable *via* NMD (*v.i.*) – nonsense mediated decay [1,6,70,71]. The first read-through agent was gentamicin, an aminoglycoside that interacts with the 40S eukaryotic ribosome subunit – at a site called the 'decoding centre'. The 40S ribosomal particle therefore contains this centre which provides surveillance for the complementarity of tRNA and mRNA to maintain the fidelity of protein translation [70,71]. For gentamicin to be effective, it needs very high concentrations, which are associated with severe side effects [6,9,10,14,70,71]. Recently Ataluren, a non-toxic bioavailable nonaminoglycoside, [PTC124], interacts with

the 60S ribosome subunit and therefore at a site distinct from the aminoglycosides [71] has been shown to produce read-through [72]. In eukaryotes, aminoglycosides cause translational misreading by binding 18S rRNA on the 40S ribosome leading to a conformational change to mimic the alteration induced by correct codon:anticodon pairing. In a sense, 'tricking' the translational apparatus at the decoding centre that all is well for insertion of near-cognate tRNA to the stop codon sequence. This mechanism generally observes the 1st and 2nd nucleotide of the codon:anticodon pair and relaxes observation of the 3rd, wobble, position. It ought to be noted that read-through drugs do not promote read-through of normal, terminal, PTCs. On a downside note though, barriers to efficient read-through include nature of the stop codon, its sequence context and nonsense mediated decay, NMD.

The most frequent substitutions causing nonsense mutations are CGA to TGA - stop (21%; 6,11) occurring *via* a methylation-mediated deamination reaction catalyzed by Activation Induced Deaminase (AID; 72). It may be then tempting to consider AID as linking epigenetics with PTC formation [73]. As part of the organization of proofreading ribosomal apparatus it is worthy to note that there is a tolerance to misincorporation and only one in ~400 misincorporations are deleterious for the protein's activity. For example, the misincorporation of an aspartate (codon: GAU/C) by near-cognateglutamate (GAA/G) both incorporate acidic amino acids. The middle and, in most cases, the first position of a codon are virtually never misread, even under error-prone conditions in the presence of aminoglycosides and are considered non-cognate for the purposes of this discussion. The genetic code lexicon is organized in such a way that selected read-through near-cognate aminoacyl-tRNAs are oftentimes chemically similar to those carried by the cognate tRNA [74]. Having said this as PTCs may arise from any part of the genetic table [8] related *vs* unrelated amino acids may be replaced. For example, CpG alteration *via* deamination to UpG: Arginine codon CGA to UGA stop – may be replaced with near match tRNA UGG for Trp- Arg is basic and Trp is aromatic nonpolar [75]. Ser: UCA/UCG mutated to UAA or UAG stops- may potentially be replaced with Tyr – both are polar residues. Structure-function may or may not be important depending on how crucial the residue that was mutated is- *viz:* active site 'pocket'. If one consults the genetic table [75] it may be seen that the genetic lexicon is organized in such as way that amino acids are encoded in a redundant fashion with observance to first and second base of the codon sequence. This is not strictly the case though for example, serine is encoded by UCA/UCG/UCC/UCU and also AGU and AGC and leucine encoded by UUA and UUG in addition to CUU and CUC and CUA and CUG. Encoding redundancy has implications with respect to mutations and the resultant amino acid that may be replaced into that site, should any one of these codons be a target for mutational event(s). The lexicon is organised too such that there is also a tendency for amino acids of similar chemical distinction, such as polar *vs* acidic *vs* basic *vs* non polar residues to be merged together when one places either 1st or 2nd nucleotide as a constant. For example, Phe, Leu, Isoleucine and Valine have nonpolar side chains and are massed together when 2nd nucleotide is held constant at a U. Such organization has impact when mutagenic point mutations occur and read-through targets these for near-cognate replacement.

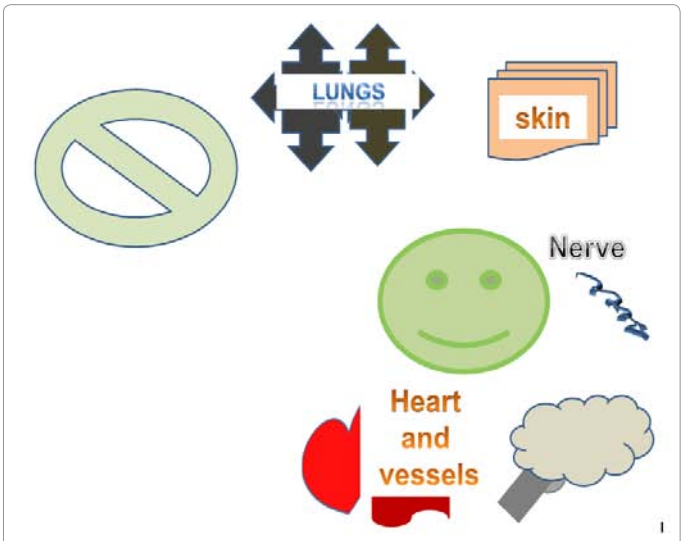

Figure 1: Outline of tissues and organs commonly affected by nonsense mediated diseases. Reading-through the nonsense codon 'stop' signal gives a green light to health.

There may be a preference too as to which amino acid is substituted as a missense read. It has been stated that glutamine is inserted at nonsense UAG or UAA, whereas UGA miscode to tryptophan [16]. Based on 3rd base 'wobble' a cysteine or tyrosine could also be incorporated frequently as near-cognate reads [75]. Again depending on how precise a replacement is then that determines the outcome of the read-through reaction and the functional outcome of the full length protein product.

Another consideration that must be considered is that when the phrase: 'truncation mutation' is stated in the literature this may also refer to a frame shifting mutation- addition or deletion of one or two nucleotide to shift the reading frame. This generally generates a PTC codon within the newly generated frame. Depending on the number of new residues inserted and their relatedness to the original amino acid residues then that determines any return to function that may be expected from read-through of an altered frame. This is inherently problematic as even reading through the first in line generated PTC in the novel frame the translation product will not match that of the original protein. So the product would be much less likely to be successful. An extended non-functional protein may also prove to be dominant negative. Altogether, these are more complex mutations to aim to rescue by simple read-through alone. For this reason, the presentation in this article is centered around simple substitution nonsense mutations.

Read-through agents

My original description of read-through for cancer [1] was inspired by the earlier work with aminoglycosdies used to induce full length beta-haemoglobin in thalassaemia derived cells [6]. This formed an early 'proof of concept' for the read-through technique. So here there was a class of antibiotics that enabled the ribosome to place in a near-cognate amino acid to the stop codon and produce full length proteins. As it turns out, sequence context of the stop codon is important as well the actual stop codon sequence itself in terms of read-through efficiency [1]. The UGA stop is more likely to be a suited read-through substrate than UAG or UAA [1]. As aminoglycosides have toxic effects such as oto- and nephro-toxicity there has been a major search for agents with less toxic effects and this has revealed Ataluren (PTC124) and numerous other compounds [76,77]. The results with Ataluren, a proven safe agent for use in clinical trials for nonsense mediated (nm) DMD [70] and nm cystic fibrosis [78] have been encouraging. These data show that read-through is 'on track' to producing significant

clinical results. Ataluren like all the read-through drugs tested to date, is somewhat codon and context sensitive [1,70] yet does little to inhibit NMD – a major issue in limiting read-through's full potential to date [70].

Importance of NMD

NMD is an issue for read-through unless the PTC is near the initiation codon [79]. The important role NMD plays may be appreciated from the following examples: eye disease/defects [80], early onset dementia [51], hereditary haemorrhagic telangiectasia [81] and Charcot Marie Tooth neuropathy [82]. Proof of concept of the value of NMD inhibition in rescuing mutant proteins levels has been shown [82,83]. Therefore an agent that is proven safe and possesses dual read-through and NMD inhibitory capacity could truly be considered a *'holy-grail'* of read-through. Amlexanox is one such agent (Enter Amlexanox).

Targeting read-through – techniques

Target biology is a key concept in appreciating the clinical power read-through may hold as the technology targets a class of mutations not just a particular gene or factor [1,84]. One must firstly identify a target protein that is associated with a particular disease in an individual in order to define mutations within the respective encoding gene that are amenable to read-through therapy. This is a central principle of *Personalised Medicine.* Identification of the target is the means by which one provides evidence that a given target selected is directly involved in the causation of a given selected disease. A '*valid target'* is one that when adjusted pharmacologically provides a significant and safely established improvement for a particular human disease over a significant time frame [84]. Validating this target is the process of showing in a clinical trial that by focusing pharmacological therapy on the target there arises a significant therapeutic benefit with safety.

In terms of nonsense read-through technology, I have previously discussed approaches to characterizing genes as targets for PTC read-through [1]. One way of approach is to utilize NMD-microarray analysis for genome-wide screen of mutated genes in any selected cancer. This combined with array-based comparative genomic hybridization may be used to reveal TS gene inactivation in cancers and in turn reveals nonsense mutations in TS in any given malignancy. Another strategy is to look for suited targets *via* NMD inhibition [1]. In this approach, NMD is pharmacologically inhibited in cultured sample of cells from a patient thus leading to stabilization of nonsense transcripts. Such drug induced differences in transcript levels may be assessed in turn by cDNA microarrays comparing disease arrays *vs* control prior to and after NMD inhibition. For a given target transcript bearing a PTC there is a given 'nonsense enrichment index' or ratio produced by NMD inhibition. Known as GINI – Gene Identification by NMD Inhibition, this approach identifies any gene harbouring nonsense codons that may well underlie a particular human disease. One of the main strengths here is that GINI does not require prior information for disease target identification. Amlexanox, the novel read-through agent which is the subject for my discussion (*v.i.*) in fact is a potent NMD inhibitor (Enter Amlexanox). By acting in such a dual fashion, Amlexanox is very well suited for both target identification *ex vivo* and to clinical read-through.

GINI has been validated for use in identifying *bona fide* nonsense mutation targets in colonic cancer as well as in Sandhoff disease [85]. This is an inherited lysosomal storage disease with an autosomal recessive pattern of transmission. It results from mutations in *HexB* gene and indeed nonsense mutations have been noted in the causative gene [86]. That study demonstrates the most frequent mutation in Sandhoff disease patients was a nonsense mutation, p.R284X, representing 29% of the alleles examined in the patient cohort. This disease is very severe and presents with devastating CNS defects due to the metabolic dysregulation. Little is available to treat these patients other than supportive means. By identifying suited read-through targets in this condition a door opens to provide actual tangible therapeutic intervention – an aim that I make throughout this article.

WES – Whole Exome Sequencing – has been used effectively in a variety of genetic diseases to delineate causative genes and therefore pinpoint targets for therapies. The rare, yet highly aggressive, salivary gland cancer – Adenoid Cystic Carcinoma (ACC) has been the subject of WES analysis [87]. Of the 312 somatic mutations discovered in this series, 14 were nonsense. Interestingly too, a missense mutation was found in ATM kinase – p.R337C. Arg 337 is highly conserved at that location and amino acid substitution mutations p.R337(S/H/C) (Serine; Histidine; Cysteine) have been reported in colonic cancer and B cell CLL [87]. This suggests that there is a risk that should a stop mutation occur at a highly conserved location in a TS then replacement of that with near- cognate (for example, Cys for Arg at p.R337) would be deleterious rather than beneficial. Such is the subtlety of the read-through mechanism that may only be examined by testing *ex vivo* and in animal models prior to investigational trials.

In the case of ACC [69] somatic truncating mutations were seen in several genes, *viz*:SUFU- encoding a component of Sonic Hedgehog and Patched signal pathway. Mutations here may predispose to cancer (Cancer stem cells and nonsense mutations) – along with the tumour suppressors: TSC1 and CYLD. Interestingly, the importance of CYLD as a suppressor gene has been reported previously [87]. Of the total number of germline CYLD mutations found in that study, 32% were substitution nonsense. The authors of that work indicated that therapeutic targeting of the CYLD regulatory axis may provide for more effective prevention and management of tumours – primary aims of read-through therapeutics. Interestingly, in ACC, somatic mutations were also found in Notch 1 and 2 [69]; [Cancer stem cell factors and nonsense mutations]. A substitution nonsense mutation in Notch 2 was found in a case of ACC along with a frame-shift truncating mutation thus forming a compound heterozygote. These results are consistent with the notion of Notch 2 as a TS or being associated with activating ('gain-of function') nonsense mutations.

Novel cancer genes may be discerned from genomic studies such as WES in rare cancer types such as ACC. Spen homolog transcriptional regulator (SPEN) is an inducible regulator identified as a cancer gene in ACC [69]. A number of substitution nonsense mutations were classified in this gene in ACC by sequence analysis [69]. Further, two nonsense mutations were found in SPEN within a 42 case ACC examination, *viz*:p.R1403X and p.Q3355X - both in solid-based histology cases. This was considered to confirm the importance of SPEN mutations in a proportion of ACC cases and in particular the part substitution nonsense mutations may play in development of the poor prognosis displayed by solid-type histology pattern. As SPEN is a Notch signaling regulator this tends to open the opportunity for Notch pathway therapeutic targeting as a potential management strategy for ACC. Read-through offers a window of opportunity in this respect.

Another dental example of WES – though in a less drastic disease state - is that for identifying target gene(s)/mutations for Amelogenesis Imperfecta/gingival hyperplasia syndrome [64]. This dental disease

though far from life-threatening, caries nonetheless considerable cosmetic burden on those afflicted as well as functional problems. It results from a genetically heterogeneous group of disorders of biomineralization causing failure of normal dental enamel – the outermost tooth layer and hardest tissue in the body. WES identified FAM20A mutations as a cause of this syndrome complex [64]. The WES approach delineated a homozygous nonsense mutation in exon 2 of FAM20A - p.Arg136X. This truncation spares the N-terminal signal sequence but prevents appropriate synthesis of the C-terminal domain. The mutant may also be considered to invoke NMD and loss of transcript, therefore protein itself [64].

In the above examples, WES has proved a powerful strategy in approaching target definition for read-through and provides that all-important link between genotype and phenotype which is at the heart of target identification.

Proof of concept - read-through

After having identified a suitable disease target the aim is then to demonstrate the ability of read-through to achieve functional rescue. This can be managed by various means through animal models of human disease and *in vitro* with cell lines derived from patients with the disease in question. Efficacy in direct human clinical trials represents, no doubt, the 'gold standard' of proof. At the present time, the evidence is compelling from a myriad of nonsense mutation targets that have been rescued with read-through that even partial restoration of function is significantly beneficial. Examples can be listed: thalassemia [6] – restoration of beta-globin in patient-derived cell lines with aminoglycoside; cystic fibrosis [78,88] – restoration of cystic fibrosis transmembrane conductance regulator (CFTR) in human clinical trials with aminoglycoside and Ataluren; Duchenne muscular dystrophy [14,70] – restoration of dystrophin in human clinical trials with Ataluren& aminoglycoside.

Notably, in cancer, APC read-through led to a reduction of ~50% in tumour burden with an *in vivo* animal model by aminoglycoside [89]. This observation for APC has support [90] in human cancer cells with aminoglycoside. For p53, read-through is functionally effective with aminoglycoside treatment of human cancer cells [91]. In Ataxia- Telangiectasia (AT), causative ATM mutations may be rescued [16,92] in human AT cell lines. Also, Rett syndrome MECP2 gene rescue [13,93] in cell lines from Rett syndrome patients treated with a novel synthetic aminoglycoside as well as in a mouse model of this human disease. Further examples are: spinal muscular atrophy SMN gene rescue [48] by aminoglycoside treatment of cell lines and by use of an *in vivo* mouse model of human disease; obesity related genes - melanocortin 4 receptor MC4R rescue [94] by aminoglycoside in cell lines; eye diseases: retinitis pigmentosa target gene rescue [95] with aminoglycoside in animal disease model; Usher syndrome target gene rescue [96] with Ataluren in cell lines and a mouse model of Usher's disease – this disease is a leading cause of deaf-blindness; lethal syndromes such as Stüve-Wiedemann target gene rescue [97] with aminoglycoside *in vitro*; metabolic disturbance target rescue [98,99] with Ataluren and aminoglycoside in patient derived cells; cardiac disease target gene rescue [4,37] in cell lines with aminoglycosides; multi-organ diseases also show rescue of target [15] with Ataluren in cell line and zebrafish rescue assay.

Importantly, functional protein levels after read-through need only to be low to be effective [100]. In DMD and CF efficiency of full length functional protein production varies from 1-25% depending on aminoglycoside, stop codon and surrounding nucleotide context [10]. In Usher syndrome [96], PTC124 gave a 2% functional protein yield and provided ~80% of binding activity assay of wild type. In retinitis pigmentosa, read-through induced ~5.3% read-through of the S334ter PTC and only ~5% reduction of abnormal truncated protein was needed to enhance photoreceptor survival [95]. In AT patients with ~5-20% normal levels of ATM protein a slower neurological progression is seen and this strongly suggests that if read-through did not attain completely normal ATM levels then that would not be altogether detrimental [16]. On a note of caution however, missense incorporation at a PTC although allowing rescue of function may alter this in subtle ways particularly if at a sensitive site of a functionally dedicated protein, for example within the heteromeric channel complex responsible for the cardiac delayed outwardly rectifying potassium current, I_{Ks} [101].

Enter amlexanox

Thus far, although proof of concept for read-through has been adequately shown, not one compound has been capable of efficient read-through of all stop codons in various contexts nor capable of inhibiting NMD. Amlexanox has changed all this and produces significant read-through of all nonsense stop codons in various contexts and inhibits NMD in cell lines [102]. Amlexanoxhas been FDA approved for more than 20 years for intraoral ulcer treatment and marketed under Aphthasol™ and is Pregnancy category B [103,104]. The safe use of Amlexanox in clinical scenarios for so many years alone speaks for the safety of this drug. Chemically it is a carboxylic acid with a simple molecular structure ($C_{16}H_{14}N_2O_4$; ~ 298 Da) and contains the bioactive 'chromenopyridine' group (Figure 2) Structurally the molecule is a non-aminoglycoside [2-amino-7-isopropyl-5-oxo-5*H*-chromeno[2,3-*b*]pyridine-3-carboxylic acid] of not dissimilar size and form to Ataluren (PTC124: 3-[5-(2-fluorophenyl)-1,2,4-oxadiazol-3-yl]benzoic acid) which is also a water soluble organic acid. It differs significantly from Ataluren though in carrying the 'chromenopyridine' nucleus.

For effective mouth ulcer treatments, it is applied directly as an ointment q.i.d. at 5%. Contraindications are few and include allergy. Adverse reactions are mild including mild stinging, nausea or gastrointestinal upset. Pharmacokinetics indicates that it is absorbed across the gastrointestinal tract and is bioavailable per that route. At a 100 mg dose of topical drug a maximum serum concentration of 120 ng/ml occurs ~$2^{1/2}$ hrs after application. Half-life of elimination *via* kidney is ~$3^{1/2}$ hrs [105]. The dental literature speaks of its safety when this topically applied anti-inflammatory-immunomodulator

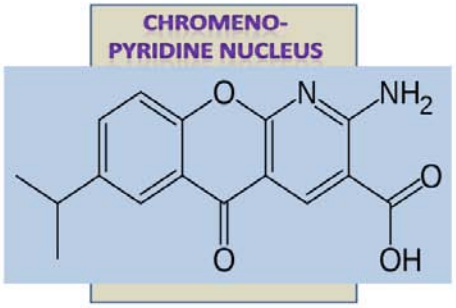

Figure 2: Molecular structure of Amlexanox highlighting the 'chromenopyridine' nucleus which occupies the central structural backbone framework of this drug.

is used for intraoral lesions in various ulcerative conditions [105-108]. Neurological studies in animals attests to the relative safety of Amlexanox as directly applied to the CNS [109].

The 'chromenopyridine nucleus' carried by Amlexanoxhas several interesting pharmacological properties [110] and has in fact been determined to represent a heterocyclic privileged medicinal scaffold and thus is highly pharmacologically relevant [111]. Compounds with the chromenopyridine nucleus have been indicated to have anticancer [112] as well as dopamine receptor antagonist properties [113]. Further, this nucleus forms a structural part of glucocorticoid receptor modulators [114]. Upon interacting with the GLUC receptor these drugs display anti-inflammatory activity comparable to that of prednisolone in suppressing cytokine production in whole blood and in rodent models of acute and chronic inflammation. Thus it is not entirely surprising that Amlexanox is entering the International arena as an exciting powerful new drug targeting diabetes and obesity [115-117]. Thus Amlexanox is being aimed to be repurposed as an important kinase inhibitor for safe therapy aiming to target obesity and insulin resistance.

In terms of its read-through capacity in nonsense mutation cell models, Amlexanox results in dystrophin localization resuming its normal configuration in ~75% cells and functional rescue of CFTR to ~20% wt [102]. Further, p53 functionality raised several fold in an *in vitro* assay [102]. Amlexanox is effective *in vitro* at the 5-25microM range which may be considered acceptable clinically as this is lower than the level of aminoglycosides needed for read-through. At the highest concentrations used (125 microM) Amlexanox is not toxic to cells and does not inhibit general translation nor alter natural NMD processes thus supporting its safety profile [102]. Post-translational modifications are maintained as demonstrated with CFTR on Amlexanoxread-through [102]. Comparatively, Amlexanox at 25 microM is ~>3 times more efficient than any of the other read-through agents such as PTC124 or aminoglycoside [102].

From the above it may be seen that it is indeed a fortuitous coincidence that Amlexanox has been discovered as an effective dual read-through and NMD inhibitor drug. Given its over 20 year success record in the field of dental care Amlexanox is certainly a fit and suitable agent to be readily tested clinically as a pharmacological agent to manage an entire myriad of nonsense mediated human diseases.

Uses for Amlexanox-based read-through technology

One is rather '*spoilt for choice*' here! I have already indicated read-through for haematological malignancies, HM [2]. Just as Gleevec offers a targeted approach in HM [118] so also does read-through - yet with the latter, targets may be diverse throughout many cancers and diseases and this remains a major strength of the technology, as mentioned [1-4]. COSMIC [8] shows frequent PTCs in key TS genes in numerous HM [2]. Read-through ought to be applied in this context and offers advantages over Gleevec which singles out for targeting a specific chromosomal translocation.

Pulmonary diseases also define useful targets [3]. These include CF and DMD and thalassemia. Very importantly carcinoma - for example, adenocarcinomas, which carry a high percentage of nonsense mutations in TS STK11, form a superb use since PTCs inactivating STK11 are implicated as causative in sporadic lung cancers – specifically adenocarcinomas [1,119]. Other TS targets are available too such as p53/ATM/Rb1/APC/NF1 [3]. Another use is in heart disease [4]. Electrical conductance genes form targets for read-through [4,37,101]. Proof of concept has been delivered in this context as has been noted (see Section: Proof of concept – read-through). Further, tackling genetic diseases with serious cardiac complications can be achieved by read-through [4,120]. Cardiomyopathy states also offer up potential read-through targets [4,121]. Nonsense mutated cardioprotectiveprosurvival factors also are envisaged targets as I have pointed out [4].

Skin and mouth conditions lend themselves particularly well to a topical read-through formulation. One particularly debilitating inherited skin condition needing to be addressed for novel therapies is epidermolysisbullosa (EB). This connective tissue disease results in a blistering condition of the skin and mucosal membranes. It results from deficiency in tethering between epidermis and underlying connective tissues and has a multigenetic basis and may present from birth – congenitally. Recessive and dominant varieties present and the degree of presentation may be relatively mild to lethal. At the very least, lesions are disfiguring with mouth lesions oftentimes affecting function [122,123]. The path to a cure for EB has been described recently as 'the long and winding road' [124]. Presently therefore no cure exists and all treatments are merely supportive. As nonsense mutations have been indicated to be oftentimes causative of this condition [125,126] read-through agents applied topically would appear ideally suited for selected nonsense mediated EB patients.

Skin cancers oftentimes present with mutations in TS genes that presumably are causatively related (Table 1) Nonsense mutations in DNA repair enzyme, ERCC5 and helicase enzyme, ERCC3, may lead to xerodermapigmentosum, a severe pre-cancerous skin condition [1]. Clearly, a preventive topical agent targeting these causative elements would be ideal – such is the opportunity afforded by read-through with Amlexanox.

In terms of dental pathology, nonsense mutations have been shown make a significant impact particularly in respect to syndromes [65,89]. Such syndromes oftentimes pose as targets for read-through as they are nonsense mutation mediated. In addition since p53 PTCs have been noted in oral premalignant & cancer lesions [127,128]. Amlexanox may well have a major role to play in prevention/managing such serious mouth-related conditions – particularly as a topical agent.

Further down the gastrointentinal tract, pancreatic cancer provides for targets *viz:* p53 and p16 TS which have a significant number of collated nonsense mutations in this disease (Table 1). A vast array of neurological conditions have targets amenable to read-through such as autism [129], schizophrenia [130], palsies [131] and eye diseases [95,96] as I have discussed earlier. Also as mentioned, one may look at ageing as a chronic disease – with a heavy accent on neurological involvement [53]. Conditions like early onset dementia [51] and Parkinson's disease [52] provide for suited targets aimed to 'place the brakes' on the premature ageing process. The question of Amlexanox negotiating the blood-brain-barrier [132,133] remains, yet, if readily surmountable, the vast array of neurological disease targets open up for read-through trials.

Another manner in which Amlexanox may be used is for the management of communicable disease. Traditionally, read-through has been described and used for non-communicable genetic diseases, such as inherited disease and sporadic cancers [1-4,6,9,10,70]. As a vast number of people around the globe are infected with either serious viral or bacterial diseases which, due to chronic damage and sequelae

have a very significant burden on human society, the potential here for read-through is of great importance to note.

Basically, communicable diseases may be divided for the sake of convenience into either viral or bacterial. In terms of bacterial-related diseases a relevant example is that of Tuberculosis (TB), a result of *Mycobacterium tuberculosis* infection. This poses as a common and very serious infection particularly in the backdrop of immune-insufficiency, a major problem in the developing world. Very recently, it has been noted that mutations in the *KatG* gene, encoding catalase/peroxidase, confer resistance to isoniazid [134]. These genetic changes play a major role in resistance to an important therapeutic agent used to manage TB. Testing for such *KatG* mutations was indicated to aid prediction of TB drug resistance [134]. Very interestingly, nonsense mutations were found to lead to high-level resistance to isoniazid with severe catalase activity loss whereas missense mutations did not demonstrate resistance nor significant catalase loss. The message here is very clear: in a serious communicable and prevalent disease such as TB, isolate genotyping for drug resistance *viaKatG* screening is paramount. This may well reveal a severe catalase disruption which is amenable to rescue *via* read-through. Combination strategies with ionized and read-through drug therapy ought to be instigated in such instances. Even a restoration of part catalase activity may be most useful in controlling what was previously a difficult to manage isolate of TB. In *Staphyloccocusaureus*, drug resistance is a significant issue. Methicillin-resistant *S. aureus* (MRSA) is a major health problem to the extent that this is now considered a leading cause of death by an infectious agent in the Western setting [135]. Glycopeptide antibiotics such as vancomycin and teicoplanin are preferred means to manage community or hospital based MRSAs [135]. Mulitigenetic causes of glycopeptides resistance were documented for a strain of *S. aureus* and three mutations were found by whole genome sequencing of such a resistant derivative strain [135]. These occurred in *stp1*, encoding a serine/threonine phosphatase and in *yjbH* encoding a post-transcriptional negative regulator of the redox/thiol challenge sensor and global transcriptional regulator, Spx. A missense mutation was found in histidine kinase sensor, VraS. It was concluded that multiple sensor/challenge paths relate to glycopeptide resistance. Read-through by simultaneously rescuing several targets could well be predicted to demonstrate redevelopment of glycopeptide sensitivity in selected *S. aureus* strains.

In viral infections, nonsense mutations play a very important role in disease progression and outcome. Hepatitis C is not able to be contained yet by vaccination and therefore poses a serious health hazard around the World. Chronic infection may lead to liver cancer. Hepatocellular carcinoma is one of the most common virus-associated cancers and a frequent cause of death Worldwide. By use of WES, a TSC1 nonsense mutation was found in a subpopulation of hepatic cancer cells in a case with Hepatitis C [136]. This shows that in this virus-associated cancer-genome genetic variation occurs with production of a notable TS nonsense substitution mutation [136]. As pointed out previously, TSC1 is an important TS gene [Neurological system: neurodevelopmental disorders] and relates to neoplasia. This shows the value of genetically screening liver cancers in HepC patients as there may be suitable targets presenting for read-through therapy. Each case must be individually examined in this respect, a valuable Personalised Medicine activity. Hepatitis B virus is also a serious and common health problem around the globe. In particular, it is interesting to note that Hepatitis B genotype-C infection may be associated with progression to hepatocellular carcinoma [137]. Substitution nonsense mutation at codon 182 of the *S* gene of genotype C was assessed and its prevalence was very significantly correlated with progressive forms of liver disease such as cirrhosis and liver cancer. It was concluded that this mutation could in itself indeed provide a causative role for liver disease progression from carrier/chronic hepatitis status [137]. This study forms a very strong argument for the use of targeted read-through therapies for individualized management of Hepatitis B patients. Rescue of gene *S* substitution nonsense mutations in this context of viral infection could well prevent, in a large proportion of cases, more advanced liver disease from arising. In a manner, this study is reminiscent of another investigation already discussed, [18]; [Importance of nonsense mutations in human disease] that shows a significantly higher prevalence of nonsense mutations in liver metastases of colonic cancer. In both examples, PTCs could well be acting as part of a cause-related mechanism for the progression of the cancer or lesion to cancer. Although the latter study is not apparently related to a viral origin there are intriguing parallels in regards nonsense mutations leading to almost a 'maturation' process of the cancerous process.

The message overall in regards communicable disease is clear and straightforward: patients require to be individually genotyped with regards their particular isolate or indeed in regards any arising pathology linked to the infectious agent. In particular, context-driven and Personalised Medicine tailored read-through therapy may well offer a most useful addition in terms of combined drug therapies within the 'fighting armamentarium' against communicable diseases. To this end, one study only has examined the potential benefits of applying read-through of nonsense mutations to communicable disease, and this involved *ex vivo* rescue of bioactive retrocyclin-1 peptide, a human viral defense protein [138]. Multiple human theta-defensin genes exist and these may produce the retrocyclins but they harbor a premature termination codon that blocks translation therefore remains as inactive pseudo genes. In order to overcome this, aminoglycoside treatment was performed and has demonstrated 'proof of concept' for communicable disease. In this respect, bioactive retrocyclins may be rescued in human cells and this may well prove to be extremely valuable in terms of their ability to ward off serious infections that are very difficult to manage through more traditional means [138]. The authors comment, quite interestingly, that in a sense, human retrocyclin-deficiency is an 'inherited disorder', yet one with an incidence of 100% - and 100% caused by a PTC forming a very profound and universal rescue target for read-through! The present-day technology is now set to literally 'reawaken' ancient inactivated anti-infectious disease defensive mechanisms. Unquestionably, the ability to reawaken this ancient molecular ancestor in order to strengthen our innate immune system to infections is one of universal great importance [138]. No doubt this shall be very significant in the future of infectious disease medicine for all people. In addition, the future for read-through with a safe, non-toxic agent for infectious disease such as Amlexanox, as I propose here, is therefore extremely bright and encouraging.

Finally, in this section, a most intriguing notion is based on examining whether read-through therapy may be used to rescue germline PTC defects prior to birth in order to avoid congenital defects. The many inherited genetic diseases [6,10] along with their associated birth defects is clearly enormous not just as forming a disability burden on the individual and family but also on society globally. Pre-birth genetic diagnosis combined with a safe and simple capsule-based therapy taken *per os* such as Amlexanox to prevent such congenital defects in nonsense mediated susceptible cases [105-110] would indeed, in its own right, be almost a 'miraculous' birth in many ways.

Cancer stem cell factors and nonsense mutations

No discussion of read-through would be complete without mentioning various nonsense mutation targets connected with cancer-related stem cells. For example, the Notch pathway has been known to developmental biologists for decades. Its role in control of stem cell proliferation has now been demonstrated for several stem cell types including hematopoietic, neural and mammary [139]. Notch signaling plays a keen role for example in normal mammary gland development by acting on both stem cells and progenitor cells. This affects self-renewal and lineage-specific differentiation. Thus dysregulated Notch expression can lead to carcinogenesis *via* deregulating self-renewal of normal stem cells. A particular branch of the Notch signaling pathway that involves the transcription factor Hes3 has been shown to regulate a number of cultured cells with cancer stem cell characteristics obtained from glioblastoma patients [140]. Hes3 is a marker for neural stem cells and is expressed in cultures of glioblastomamultiforme (GM). Thus Hes3 forms a cancer therapy target since this marker relates to stem cells in, for example, GM. Hes3 is not only a relevant target for the elusive cancer stem cell population of GM but also for possibly other tumours as well [141-143].

Notch has been also portrayed as a TS [144] and protective agent [145]. Notch receptors signal in response to ligands on neighbouring cells regulating lineage selection and developmental patterning. When deregulated, Notch allows microenvironmental communication to break down, a feature in cancer and perhaps too in other diseases. Notch mutations include substitution nonsense mutations that block appropriate signaling functions [146]. In terms of exercising a protective-type role, it has been found that disrupting mutations of Notch 3 lead to a hereditary adult onset condition of stroke and vascular dementia - CADASIL [143]. Although this study pinpoints mis-sense mutations as being disruptive it does indicate that the Notch signaling axis is crucial in maintaining central nervous system homeostasis. Further, it tends to indicate that as the mis-sense mutants were spread out over seven exons in Notch then there are several regions of structural-functional sensitivity. It is quite conceivable that further Notch 3 disrupting mutations will be isolate in time and that a percentage of these may well be PTC events. Read-through would have to pay attention to the fine details of function structure to be clinically meaningful in such therapeutic scenarios.

Oncogenic - gain of function type - mutations in Notch occur not uncommonly in human leukaemia [145]. Hence, there appears a dual role for Notch in cancer *viz:* both as TS and as an oncogenic factor. In head and neck squamous cell carcinoma, a very significant proportion of Notch inactivating mutations are substitution nonsense (25%) [146] suggesting a TS role in this context. Interestingly, sporadic nonsense mutations in Notch have been noted in mouth cancer as well as skin cancers at approximately the same % (Table 1).

As Notch1 signaling leads to a maintained stem cell population, dysregulated activation of Notch may produce cancers, reflecting its oncogenic side [147]. In lung cancer, downregulation of Notch leads to reduction in cancer stem-like CD44+/CD24- cells in that tumour consistent with its oncogenic role in that particular tumour context. Further, in respect to oncogenic 'functional gain' a nonsense mutation in Notch has been found in a young patient with myeloid/NK cell precursor acute leukemia – MNKL [145]. The mutation, p.S2471X, occurred in the PEST domain of Notch1 in this patient. The presence of the Notch1 activating mutation in MNKL may suggest a causative role in this cancer.

It ought to be noted that PEST domain is involved in protein stability and this acts as a signal peptide for protein degradation [148]. Thus there is an inverse correlation of PEST regions with intracellular stability. By removal of PEST by nonsense truncation, the Notch factor was stabilized and hence paradoxically 'activated' – producing a gain of function. Notch1 is involved in normal haematopoiesis to maintain the haematopoietic stem cell population and stem cell self-renewal and determination of lymphoid progenitor cell fate. So deregulated activation of the Notch1 signaling pathway results in transformation of T-cell progenitors and generally results in human T-ALL. About half of human T-ALL has activating mutations in the extracellular and terminal PEST domain of Notch1. Such mutations are not seen in controls nor in patients with precursor B cell-ALL and appears rather specific for T-ALL. In fact, small molecular inhibitors which block Notch1 activation *in vitro* and *in vivo* may be predicted to possess anti-leukemic effects for T-ALL. Read-through, by rescuing any occurring nonsense mutations in the PEST domain of Notch would also be predicted to be a viable therapeutic strategy for T-ALL. Overall, there is no doubt that dysregulation of Notch expression is a key factor in destabilizing the stem cell environment though Notch mutations lead to this in a variable fashion.

Therapies for the Notch pathway have been proposed of late [149]. These include using gamma secretase inhibitors to inhibit Notch signaling but these have significant adverse reactions [149]. Antibody techniques too are proposed [149]. Frequencies of mutations differ in the four Notch genes. It has been noted that 4.7% of several commonly found solid-based tumours carry Notch 1 nonsense or mis-sense mutations. Notch 2 and 3 contained ~1.5% of these disrupting mutations with Notch 4 presenting with far fewer [150]. A key point is made by the authors [150] that targeting Notch for cancer therapy in regards solid-based tumours is fundamentally hampered by confusion over whether Notch signaling may be either acting as a TS or in an activator 'gain of function' capacity. Doubtlessly, Notch mutational targeting in cancer is very important nonetheless but is very much a context dependent situation. Various activators and inhibitors of Notch are in early phase clinical development for solid cancers, but of course, as stated, the particular functioning of Notch in each context must be assessed before benefits may be gained. Read-through with a safe and already accepted agent such as Amlexanox (Enter Amlexanox) would be also very applicable to the Notch story.

All-in-all, it is comforting to realize that either side of the Notch character may form a target for read-through *viz:* its TS nature, with nonsense substitution mutation read-through or conversely, reverting activating 'gain of function' nonsense mutations - for example those presenting in the Notch PEST domain. As such, I propose read-through ought to be added to the armamentarium of Notch-based therapies for cancer.

Another important cancer stem cell mechanism is that of the Hedgehog pathway which plays a major role in controlling stem cell fate, self-renewal and maintenance along with drug resistance. Hedgehog contributes to survival of tumour progenitor cells and interacts with the PTCH1 receptor [151]. It is noted too that PTCH may act as a TS and is frequently mutated certain cancers, for example BCC (Table 1). In the case of adenoid cystic carcinoma, a rare but lethal salivary gland malignancy, [69], somatic truncating mutations have been detected in several genes. These include SUFU, a TS encoding a component of Hedgehog and PTCH signal pathway. Mutations in this factor may predispose to cancer. Therefore, there is a confluence

between stem cell pathways and TS pathways. Nonsense mutations in PTCH clearly have a pro-cancer role to play in addition to SUFU mutations – not unsurprisingly as they are considered part of the same signal cascade. Rescue by nonsense read-through of these TS may only be seen as beneficial in these various contexts by moderating the stem cell prosurvival signaling axis.

Finally, in regards mammary gland carcinoma, stem cells may materially contribute to cardinal features of this disease, *viz:* growth, recurrence, metastases and drug resistance [152]. The aim is therefore to eliminate such cells. Hedgehog and Notch are central pathways in this context, governing tumour formation and cancer stem cell renewal. But the point is made [152] that as these signaling pathways play a central part in normal mammary gland development then specific targeting of alterations in these signaling pathway receptors needs to be much selected. Read-through may offer that type of selection by simply 'rescuing' what may only be considered to be normal function, for example with Notch nonsense TS mutations and 'gain of function' mutations. Again, trialing this technology in such selected instances of mammary gland carcinoma and a multitude of other cancers that form targets in this regard is my central suggested aim in respect to cancer stem cell modulation.

General discussion and conclusion

Read-through clearly has a marvelous potential to rescue disease-causing mutations from many different disease states. Key to the success of this strategy is the availability of a safe and accepted agent that demonstrates excellent 'proof of principle'. To this end, my estimation is that the next generation of read-through drugs is here, with the advent of Amlexanox. This drug is just now re-entering the International Arena as an exciting powerful 'off patent' novel drug for diabetes and obesity (Enter Amlexanox). Now it readily offers itself up as a potent read-through agent [104]. Amlexanox has a further benefit of inhibiting the angiogenic and mitogenic activity of FGF1 [153] - thus supporting its use as an anti-cancer therapeutic in its own right. It is tempting to model this agent along the lines of other medications that offer multiple benefits, such as Statins, which also have an immunomodulatory role like Amlexanox [154].

The precise mechanism how Amlexanox may induce read-through and NMD inhibition is not yet clarified. Another feature not understood is whether it may cross the blood brain barrier to any significant degree. Although many technologies are rapidly developing in that area [132,133] the field is still an active research one. Clearly, in the longer term, a definition of these properties would be required to appreciate its true read-through potential. Nonetheless, as Amlexanox has been in the pharmaceutical marketplace for over two decades with minimal adverse reports then this speaks of its readiness to be clinically trialed for a broad range of genetic diseases including cancer. There is no doubt that the search for suited bioavailable and proven safe read-through agents is becoming a *'holy grail'* in the field. Very recent studies aimed at the development of novel read-through drugs [94] have used ATM cell lines from patients with the three different stop codons in these TS. Results showed good tolerance to the cells. Unfortunately, it shall be a lengthy and arduous process to gain FDA acceptance for clinical application.

Cancer

Doubtlessly, many read-through targets remain as yet undiscovered. This point is made in respect to mammary gland carcinoma [155]. Authors indicate that there are indeed a multitude of genetic alterations involved in mammary carcinoma development from oncogene activation to loss of TS genes and many TS remain to be actually identified. A nonsense mutation in the L3MBTL4 gene (Lethal(3) Malignant Brain Tumour-Like Protein 4) was found. This gene localizes to a region on 18p along with other TS candidates. Notably, L3MBTL4 is a polycomb group protein which maintains a transcriptionally repressive status. These proteins may remodel chromatin structure such that epigenetic silencing of genes occurs. Other members such as Bmi1 polycomb promotes stem cell self-renewal by repressing TS CDKN2A induced senescence [156]. This therefore suggests that polycomb proteins function to repress TS so acting in an ambivalent fashion in regards cancer – particularly in the stem cell context. Mammary carcinomas harbouring such 18p alterations correspond with a severe clinical phenotype [155]. By read-through therapy the inactive polycomb member L3MBTL4 may be rescued thereby reverting the severity of this particular mammary carcinoma type. Other effects such as maintaining a stem cell state *via* the polycomb axis would have to be assessed in respect to this particular type of solid-based cancer – though its effects as primary TS may be more relevant in determining outcome. Studying this and other such targets in animal models for read-through would provide evidence for the true degree of benefit that read-through might be anticipated to have in such an instance.

Already, WES has revealed novel cancer related genes in such rare cancer types such as ACC [69]. Thus, many cancers remain to be further analysed to reveal novel regulatory targets for read-through. Nonsense mutations are also related to therapy resistance in cancer [157]. Checkpoint gene CHEK2 nonsense mutations have been found to predict resistance to chemotherapy in tumours harbouring a wild type p53. CHEK2 is a downstream checkpoint protein in the p53 pathway. The authors note that alterations affecting the so-called 'p53 pathway' may be considered as playing a central role in chemotherapy resistance in cancer. In this respect it acts as a 'beacon' directing attention to key gene cascades – in this instance forming a good target for read-through therapy.

Nonsense mutations play an important role too in determining cancer recurrence and may be a molecular marker to predict that particular behaviour and to distinguish from more indolent counterparts. For example, mutations in isocitrate dehydrogenase 1 (IDH1) relate to cancer formation [158]. Isocitrate dehydrogenases catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate. The isoforms IDH1/2 use $NADP^+$ as cofactor in lieu of NAD^+ which is used by the well known mitochondrial isoform IDH3 catalyzing the third step of the citric acid cycle. Nonsense mutations have been characterized in IDH2. Authors suggested that IDH1/2 testing is valuable in distinguishing tumours, *viz:* glioblastomas/gliomas from the more benign counterpart, ganglioglioma. Mutations in these isoforms correlated with greater degree of recurrence and overall malignant behaviour pattern [158]. Hence nonsense mutations appear to play a role in this context and understanding that may open doors to revealing useful read-through targets to prevent degeneration of neural growths into frank cancer [159].

It has been shown that in cells derived from patients with high-grade serous ovarian cancer, p53 nonsense mutations were present [160]. These cancers demonstrated a recurrence pattern and, in instances, chemotherapy resistance. Clearly, nonsense mutations in a key TS, *viz:* p53, are related to recurrence and other features of cancers that provide for survival enhancement within their host. Along

these lines, as I noted earlier [18] nonsense mutations in p53 appear to correlate with tumour progression *viz:* within metastasis in colonic carcinoma. Genotyping cancers becomes complex when one considers that there are considerable genetic developments that occur in the 'cancer process' from precancer to metastasis. Nonetheless, probing for nonsense changes is important as these may relate to this process. All-in-all these observations correlate with p53 as an excellent target for read-through-based therapies, for example, with Amlexanox. Such a therapy may make all the difference to the longer term survival of a patient carrying cancer with such TS mutations.

Heart disease

Moving to heart disease, the other major cause of morbidity and mortality around the World it may be readily seen that this too is indeed relevant to read-through therapeutics. It has been noted that the p.R1141X mutation of the Abcc6 gene is a significant risk factor for development of coronary artery disease – CAD [56]. Pseudoxanthomaelasticum (PE) the disease resulting from Abcc6 disruption oftentimes presents with early onset CAD and is a model in itself for premature arterial wall calcification [161]. Importantly for PE, read-through has already demonstrated proof of concept in a read-through model in zebrafish with Ataluren [15]. Further, and importantly, Abcc6 is a prosurvival factor involved in cardioprotection- that is, cardiomyocyte survival after ischaemia/reperfusion [162]. Therefore restoration of activity of this protein is certainly potentially beneficial in terms of heart health directly as well as vascular integrity [4]. Nonetheless, one comment ought to be made though, since as I have observed previously [163,164] prosurvival factors in heart match those in cancer. Thus by restoration of functionally inactive Abcc6, the goal of read-through, this would have an impact on cancer therapy resistance. In fact Abcc6, is part of the family of ATP-binding cassette transporters and are multidrug resistance proteins. These proteins transport out of malignant cells chemotherapy compounds and are responsible for, to a large part, cancer therapy resistance [165]. As such, in restoring Abcc6 activity one ought to be aware of this property. To what extent read-through of nonsense mutated Abcc6 may have in producing cancer therapy resistance in the backdrop of a patient with cancer as a comorbidity is not simple to gauge. Nonetheless, the specific indication of use for read-through of Abcc6 needs to be clearly outlined- truly a case for *Personalised Medicine*.

Other targets are of relevance in vascular diseases. For example, by WES, a causative nonsense mutation has been found in the TGFB2 gene resulting in aortic aneurysms [166]. Significantly, Notch nonsense mutations have been determined that correlate with aortic valve disease in a large family transmitted in an autosomal-dominant fashion [167]. This links the Notch axis involved in stem cell homeostasis with a tangible disease process *via* nonsense mutations.

Neurology

One of the interesting aspects of the discussion surrounding read-through applications concerns itself with ageing. By examining ageing as a chronic disease one can see that many premature ageing conditions relate to neurological molecular breakdown in various defined targets – as illustrated by early onset Alzheimer's and Parkinson's diseases and fronto-temporal lobar dementia (Neurodegeneration – ageing). Many of these diseases marking premature ageing have nonsense mutations that may form suited therapeutic targets for read-through. Another interesting aspect arising from examining neurological read-through targets comes from spinal muscular atrophy (SMA) – a relatively common genetic muscular wasting disease linked to SMN1 gene mutations. It is inherited as autosomal recessive and varies with time of onset either early in infancy/congenitally or later in life as a devastating affliction. Read-through in mouse models of SMA has shown some benefit with aminoglycoside [48]. Doubtlessly read-through has a very bright future for managing a nonsense-mediated subgroup of SMA patients. Nonsense mutational disruption of a kinase that is required for nuclear membrane formation [47] is considered additionally causatively related to SMA. This suggests that nuclear envelope integrity relates to neuronal homeostasis/maintenance. Further, nuclear lamina has been noted to provide a structural support for the nucleus. The laminas are the constituents of this lamina. Interestingly, a lamina nonsense mutant from a patient with a diagnosed 'laminopathy' lead to altered nuclear envelope dynamics [167]. Nonsense mutations in the lamins also correlate with cardiomyopathy [168]. Clearly, the nuclear membrane integrity is vital in many diseases including the devastating and untreatable SMA. It is hoped that read-through shall be able to rescue normal nuclear membrane dynamics in selected nonsense-mediated cases and be able to offer hope to patients with serious conditions arising from such pathology.

Prevention vs management

As I have suggested before [162], prevention is far preferable to attempts at a cure! In respect to read-through, this is a highly applicable yet attainable truism. Many diseases that are inherited may be diagnosed *via* molecular techniques very early on, even from pre-birth. This allows a major window of opportunity to apply read-through therapy to those cases that are nonsense-mediated, which, from the above presentation, can be seen to represent a major proportion of diseases. Certain syndromes develop later in life, for example, SMA, and can be diagnosed early on and prophylaxis *via* read-through may be applied to targeted cases – a very practical and beneficial *Personalised Medicine* indeed.

In cancer, the evidence suggests that inherited predisposition plays a significant role in a proportion of cancers [9]. In these cases, prophylaxis with read-through in selected cases that may be targeted would prove a major leap forward in preventive medicine in itself.

In terms of management, when a disease phenotype becomes apparent it may still be a suited target for read-through therapy, for example in nonsense mediated cancers as I suggested originally in this field [1]. Doubtlessly, many other conditions may be rescued *via* read-through and cystic fibrosis and DMD trials have already demonstrated that this is certainly a strong possibility. My proposition in the present article is to draw attention towards Amlexanox therapy for both management and prophylaxis given its strong read-through capacity representing the dual NMD inhibitory role it offers. Further, its long 'track record' of safety is very encouraging indeed.

Final Comments

For my own viewpoint, Amlexanox 'ticks all the boxes' for clinical trials aimed at 'running the red light' for read-through in the vast number of human diseases related to nonsense mutations. The concept of actual *Personalised Medicine* aimed at preventing many debilitating conditions is most attractive in itself in addition to more effective management strategies. Importantly, use of read-through has a marvelous tangible potential to make a very significant impact in the prevention and management of infectious diseases. This opens a whole new vista up for fruitful clinical exploration that has already shown 'proof of concept'. Downsides of read-through appear very few,

although longer term risk of formation of resistance to read-through is a thought – this potential certainly ought not to deter in any way from trialing Amlexanox. In the final analysis, any benefits attained from its use will be most welcome by many millions of persons around the World who have diseases related to nonsense mutations. It is they who shall be the onesto reap the many benefits to be derived from diving into the era of targeted *Personalised Medicine*.

References

1. Loudon JA (2010) Cancer: a novel "no-nonsense" approach to treatment. Oncol Res 19: 55-66.
2. John A. Loudon (2013) Ataluren – time for a 'no-nonsense' approach to haematological malignancies. Hematology & Leukemia 1:2
3. Loudon JA (2013) Ataluren: a 'no-nonsense' approach for pulmonary diseases. Pulm Pharmacol Ther 26: 398-399.
4. Loudon JA (2013) Ataluren: time for a 'no-nonsense' approach to heart diseases. Cardiovasc Drugs Ther 27: 181-182.
5. Human Genome Mutation Database.
6. Salvatori F, Breveglieri G, Zuccato C, Finotti A, Bianchi N, et al. (2009) Production of beta-globin and adult hemoglobin following G418 treatment of erythroid precursor cells from homozygous beta(0)39 thalassemia patients. Am J Hematol 84: 720-728.
7. Goldmann T, Overlack N, Moller F, Belakhov V, van Wyk M, et al. (2012) A comparative evaluation of NB30, NB54 and PTC124 in translational read-through efficacy for treatment of an USH1C nonsense mutation. EMBO Mol Med 4: 1186-1199.
8. Catalogue of Somatic Mutations in Cancer – COSMIC
9. Bordeira-Carriço R, Pego AP, Santos M, Oliveira C (2012) Cancer syndromes and therapy by stop-codon readthrough. Trends Mol Med 18: 667-678.
10. Keeling KM, Bedwell DM (2011) Suppression of nonsense mutations as a therapeutic approach to treat genetic diseases. Wiley Interdiscip Rev RNA 2: 837-852.
11. Mort M, Ivanov D, Cooper DN, Chuzhanova NA (2008) A meta-analysis of nonsense mutations causing human genetic disease. Hum Mutat 29: 1037-1047.
12. Kerem E (2004) Pharmacologic therapy for stop mutations: how much CFTR activity is enough? CurrOpinPulm Med 10: 547-552.
13. Brendel C, Belakhov V, Werner H, Wegener E, Gartner J, et al. (2011) Readthrough of nonsense mutations in Rett syndrome: evaluation of novel aminoglycosides and generation of a new mouse model. J Mol Med (Berl) 89: 389-398.
14. Govoni A, Magri F, Brajkovic S, Zanetta C, Faravelli I, et al. (2013) Ongoing therapeutic trials and outcome measures for Duchenne muscular dystrophy. Cell Mol Life Sci.
15. Zhou Y, Jiang Q, Takahagi S, Shao C, Uitto J (2013) Premature Termination Codon Read-Through in the ABCC6 Gene: Potential Treatment for Pseudoxanthoma Elasticum. J Invest Dermatol.
16. Lai CH, Chun HH, Nahas SA, Mitui M, Gamo KM, et al. (2004) Correction of ATM gene function by aminoglycoside-induced read-through of premature termination codons. Proc Natl Acad Sci U S A 101: 15676-15681.
17. Nikitin PA, Yan CM, Forte E, Bocedi A, Tourigny JP, et al. (2010) An ATM/Chk2-mediated DNA damage-responsive signaling pathway suppresses Epstein-Barr virus transformation of primary human B cells. Cell Host Microbe 8: 510-522.
18. Miyaki M, Iijima T, Yasuno M, Kita Y, Hishima T, et al. (2002) High incidence of protein-truncating mutations of the p53 gene in liver metastases of colorectal carcinomas. Oncogene 21: 6689-6693.
19. Katoh H, Shibata T, Kokubu A, Ojima H, Kosuge T, et al. (2006) Genetic inactivation of the APC gene contributes to the malignant progression of sporadic hepatocellular carcinoma: a case report. Genes Chromosomes Cancer 45: 1050-1057.
20. Bonnet C, Ali Khan A, Bresso E, Vigouroux C, Beri M, et al. (2013) Extended spectrum of MBD5 mutations in neurodevelopmental disorders. Eur J Hum Genet.
21. Kong XF, Bousfiha A, Rouissi A, Itan Y, Abhyankar A, et al. (2013) A novel homozygous p.R1105X mutation of the AP4E1 gene in twins with hereditary spastic paraplegia and mycobacterial disease. PLoS One 8: e58286.
22. Boda B, Alberi S, Nikonenko I, Node-Langlois R, Jourdain P, et al. (2004) The mental retardation protein PAK3 contributes to synapse formation and plasticity in hippocampus. J Neurosci 24: 10816-10825.
23. Kreis P, Thevenot E, Rousseau V, Boda B, Muller D, et al. (2007) The p21-activated kinase 3 implicated in mental retardation regulates spine morphogenesis through a Cdc42-dependent pathway. J Biol Chem 282: 21497-21506.
24. Xu X, Zhang L, Tong P, Xun G, Su W, et al. (2013) Exome sequencing identifies UPF3B as the causative gene for a Chinese non-syndrome mental retardation pedigree. Clin Genet 83: 560-564.
25. Vodopiutz J, Zoller H, Fenwick AL, Arnhold R, Schmid M, et al. (2013) Homozygous SALL1 mutation causes a novel multiple congenital anomaly-mental retardation syndrome. J Pediatr 162: 612-617.
26. Yimenicioglu S, Yakut A, Karaer K, Zenker M, Ekici A, et al. (2012) A new nonsense mutation in the NF1 gene with neurofibromatosis-Noonan syndrome phenotype. Childs NervSyst 28: 2181-2183.
27. Bottillo I, Ahlquist T, Brekke H, Danielsen SA, van den Berg E, et al. (2009) Germline and somatic NF1 mutations in sporadic and NF1-associated malignant peripheral nerve sheath tumours. J Pathol 217: 693-701.
28. Evans GR, Lloyd SK, Ramsden RT (2011) Neurofibromatosis type 2. AdvOtorhinolaryngol 70: 91-98.
29. Vrtel R, Verhoef S, Bouman K, Maheshwar MM, Nellist M, et al. (1996) Identification of a nonsense mutation at the 5' end of the TSC2 gene in a family with a presumptive diagnosis of tuberous sclerosis complex. J Med Genet 33: 47-51.
30. Niida Y, Lawrence-Smith N, Banwell A, Hammer E, Lewis J, et al. (1999) Analysis of both TSC1 and TSC2 for germline mutations in 126 unrelated patients with tuberous sclerosis. Hum Mutat 14: 412-422.
31. Hamdan FF, Daoud H, Rochefort D, Piton A, Gauthier J, et al. (2010) De novo mutations in FOXP1 in cases with intellectual disability, autism, and language impairment. Am J Hum Genet 87: 671-678.
32. Berkel S, Marshall CR, Weiss B, Howe J, Roeth R, et al. (2010) Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nat Genet 42: 489-491.
33. Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, et al. (2012) De novo mutations revealed by whole-exome sequencing are strongly associated with autism. Nature 485: 237-241.
34. Trudeau MM, Dalton JC, Day JW, Ranum LP, Meisler MH (2006) Heterozygosity for a protein truncation mutation of sodium channel SCN8A in a patient with cerebellar atrophy, ataxia, and mental retardation. J Med Genet 43: 527-530.
35. Generalized epilepsy with febrile seizures plus.
36. Rilstone JJ, Coelho FM, Minassian BA, Andrade DM (2012) Dravet syndrome: seizure control and gait in adults with different SCN1A mutations. Epilepsia 53: 1421-1428.
37. Teng S, Gao L, Paajanen V, Pu J, Fan Z (2009) Readthrough of nonsense mutation W822X in the SCN5A gene can effectively restore expression of cardiac Na+ channels. Cardiovasc Res 83: 473-480.
38. Lignani G, Raimondi A, Ferrea E, Rocchi A, Paonessa F, et al. (2013) Epileptogenic Q555X SYN1 mutant triggers imbalances in release dynamics and short-term plasticity. Hum Mol Genet 22: 2186-2199.
39. CastrÃ©n M, Gaily E, Tengstrom C, Lahdetie J, Archer H, et al. (2011) Epilepsy caused by CDKL5 mutations. Eur J Paediatr Neurol 15: 65-69.
40. Loureiro JL, Miller-Fleming L, Thieleke-Matos C, Magalhaes P, Cruz VT, et al. (2009) Novel SPG3A and SPG4 mutations in dominant spastic paraplegia families. Acta Neurol Scand 119: 113-118.
41. Burk K, Dalski A, Purmann S, Zuhlke C (2005) Detection of a novel SPG4-nonsense-mutation in a large German pedigree with pure spastic paraplegia. J Neurol 252: 371.

42. Devon RS, Helm JR, Rouleau GA, Leitner Y, Lerman-Sagie T, et al. (2003) The first nonsense mutation in alsin results in a homogeneous phenotype of infantile-onset ascending spastic paralysis with bulbar involvement in two siblings. Clin Genet 64: 210-215.

43. Verschuuren-Bemelmans CC, Winter P, Sival DA, Elting JW, Brouwer OF, et al. (2008) Novel homozygous ALS2 nonsense mutation (p.Gln715X) in sibs with infantile-onset ascending spastic paralysis: the first cases from northwestern Europe. Eur J Hum Genet 16: 1407-1411.

44. Hadano S, Hand CK, Osuga H, Yanagisawa Y, Otomo A, et al. (2001) A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. Nat Genet 29: 166-173.

45. Tamer Z, Bertelsen B, Gredal O, Magyari M, Nielsen KC, et al. (2012) Novel heterozygous nonsense mutation of the OPTN gene segregating in a Danish family with ALS. Neurobiol Aging 33: 208.

46. Brichta L, Garbes L, Jedrzejowska M, Grellscheid SN, Holker I, et al. (2008) Nonsense-mediated messenger RNA decay of survival motor neuron 1 causes spinal muscular atrophy. Hum Genet 123: 141-153.

47. Renbaum P, Kellerman E, Jaron R, Geiger D, Segel R, et al. (2009) Spinal muscular atrophy with pontocerebellar hypoplasia is caused by a mutation in the VRK1 gene. Am J Hum Genet 85: 281-289.

48. Heier CR, DiDonato CJ (2009) Translational read through by the aminoglycoside geneticin (G418) modulates SMN stability in vitro and improves motor function in SMA mice in vivo. Hum Mol Genet 18: 1310-1322.

49. Pottier C, Hannequin D, Coutant S, Rovelet-Lecrux A, Wallon D, et al. (2012) High frequency of potentially pathogenic SORL1 mutations in autosomal dominant early-onset Alzheimer disease. Mol Psychiatry 17: 875-879.

50. van der Zee J, Urwin H, Engelborghs S, Bruyland M, Vandenberghe R, et al. (2008) CHMP2B C-truncating mutations in frontotemporal lobar degeneration are associated with an aberrant endosomal phenotype in vitro. Hum Mol Genet 17: 313-322.

51. Ward ME, Miller BL (2011) Potential mechanisms of progranulin-deficient FTLD. J Mol Neurosci 45: 574-582.

52. Hedrich K, Hagenah J, Djarmati A, Hiller A, Lohnau T, et al. (2006) Clinical spectrum of homozygous and heterozygous PINK1 mutations in a large German family with Parkinson disease: role of a single hit? Arch Neurol 63: 833-838.

53. Alcedo J, Flatt T, Pasyukova EG (2013) The role of the nervous system in aging and longevity. Front Genet 4: 124.

54. Wang L, Guo DC, Cao J, Gong L, Kamm KE, et al. (2010) Mutations in myosin light chain kinase cause familial aortic dissections. Am J Hum Genet 87: 701-707.

55. Song YH, Kim GH, Yoo HW, Kim JB (2012) Novel de novo nonsense mutation of FBN1 gene in a patient with Marfan syndrome. J Genet 91: 233-235.

56. Koblos G, Andrikovics H, ProhÃ¡szka Z, Tordai A, VÃ¡radi A, et al. (2010) The R1141X loss-of-function mutation of the ABCC6 gene is a strong genetic risk factor for coronary artery disease. Genet Test Mol Biomarkers 14: 75-78.

57. Ajmal M, Ahmed W, Akhtar N, Sadeque A, Khalid A, et al. (2011) A novel pathogenic nonsense triple-nucleotide mutation in the low-density lipoprotein receptor gene and its clinical correlation with familial hypercholesterolemia. Genet Test Mol Biomarkers 15: 601-606.

58. Anderson JL, Habashi J, Carlquist JF, Muhlestein JB, Horne BD, et al. (2000) A common variant of the AMPD1 gene predicts improved cardiovascular survival in patients with coronary artery disease. J Am Coll Cardiol 36: 1248-1252.

59. Kharrat M, Trabelsi S, Chaabouni M, Maazoul F, Kraoua L, et al. (2010) Only two mutations detected in 15 Tunisian patients with 11Î²-hydroxylase deficiency: the p.Q356X and the novel p.G379V. Clin Genet 78: 398-401.

60. Bouligand J, Delemer B, Hecart AC, Meduri G, Viengchareun S, et al. (2010) Familial glucocorticoid receptor haploinsufficiency by non-sense mediated mRNA decay, adrenal hyperplasia and apparent mineralocorticoid excess. PLoS One 5: e13563.

61. Al-Daghri NM, Clerici M, Al-Attas O, Forni D, Alokail MS, et al. (2013) A nonsense polymorphism (R392X) in TLR5 protects from obesity but predisposes to diabetes. J Immunol 190: 3716-3720.

62. Noack B, GÃrgens H, Schacher B, Puklo M, Eickholz P, et al. (2008) Functional Cathepsin C mutations cause different Papillon-LefÃ¨vre syndrome phenotypes. J Clin Periodontol 35: 311-316.

63. Nitta H, Wara-Aswapati N, Lertsirivorakul J, Nakamura T, Yamamoto M, et al. (2005) A novel mutation of the cathepsin C gene in a thai family with Papillon-Lefevre syndrome. J Periodontol 76: 492-496.

64. O'Sullivan J, Bitu CC, Daly SB, Urquhart JE, Barron MJ, et al. (2011) Whole-Exome sequencing identifies FAM20A mutations as a cause of amelogenesisimperfecta and gingival hyperplasia syndrome. Am J Hum Genet 88: 616-620.

65. Lin WD, Lin SP, Wang CH, Tsai Y, Chen CP, et al. (2011) RUNX2 mutations in Taiwanese patients with cleidocranial dysplasia. Genet Mol Biol 34: 201-204.

66. Ballew BJ, Yeager M, Jacobs K, Giri N, Boland J, et al. (2013) Germline mutations of regulator of telomere elongation helicase 1, RTEL1, in Dyskeratosiscongenita. Hum Genet 132: 473-480.

67. Pan S, Xu LL, Sun LS, Li TJ (2009) Identification of known and novel PTCH mutations in both syndromic and non-syndromickeratocystic odontogenic tumors. Int J Oral Sci 1: 34-38.

68. Kijima C, Miyashita T, Suzuki M, Oka H, Fujii K (2012) Two cases of nevoid basal cell carcinoma syndrome associated with meningioma caused by a PTCH1 or SUFU germline mutation. Fam Cancer 11: 565-570.

69. Stephens PJ, Davies HR, Mitani Y, Van Loo P, Shlien A, et al. (2013) Whole exome sequencing of adenoid cystic carcinoma. J Clin Invest 123: 2965-2968.

70. Bidou L, Allamand V, Rousset JP, Namy O (2012) Sense from nonsense: therapies for premature stop codon diseases. Trends Mol Med 18: 679-688.

71. Linde L, Kerem B (2008) Introducing sense into nonsense in treatments of human genetic diseases. Trends Genet 24: 552-563.

72. Fritz EL, Papavasiliou FN (2010) Cytidinedeaminases: AIDing DNA demethylation? Genes Dev 24: 2107-2114.

73. Schmitz KM, Petersen-Mahrt SK (2012) AIDing the immune system-DIAbolic in cancer. SeminImmunol 24: 241-245.

74. Di Giacco V, MÃ¡rquez V, Qin Y, Pech M, Triana-Alonso FJ, et al. (2008) Shine-Dalgarno interaction prevents incorporation of noncognate amino acids at the codon following the AUG. ProcNatlAcadSci U S A 105: 10715-10720.

75. The Genetic Code

76. Du L, Damoiseaux R, Nahas S, Gao K, Hu H, et al. (2009) Nonaminoglycoside compounds induce readthrough of nonsense mutations. J Exp Med 206: 2285-2297.

77. Nudelman I, Rebibo-Sabbah A, Cherniavsky M, Belakhov V, Hainrichson M, et al. (2009) Development of novel aminoglycoside (NB54) with reduced toxicity and enhanced suppression of disease-causing premature stop mutations. J Med Chem 52: 2836-2845.

78. Sermet-Gaudelus I, Boeck KD, Casimir GJ, Vermeulen F, Leal T, et al. (2010) Ataluren (PTC124) induces cystic fibrosis transmembrane conductance regulator protein expression and activity in children with nonsense mutation cystic fibrosis. Am J RespirCrit Care Med 182: 1262-1272.

79. Inacio A, Silva AL, Pinto J, Ji X, Morgado A, et al. (2004) Nonsense mutations in close proximity to the initiation codon fail to trigger full nonsense-mediated mRNA decay. J Biol Chem 279: 32170-32180.

80. Yahyavi M, Abouzeid H, Gawdat G, de Preux AS, Xiao T, et al. (2013) ALDH1A3 loss of function causes bilateral anophthalmia/microphthalmia and hypoplasia of the optic nerve and optic chiasm. Hum Mol Genet 22: 3250-3258.

81. Govani FS, Giess A, Mollet IG, Begbie ME, Jones MD, et al. (2013) Directional next-generation RNA sequencing and examination of premature termination codon mutations in endoglin/hereditary haemorrhagic telangiectasia. MolSyndromol 4: 184-196.

82. Xu WY, Gu MM, Sun LH, Guo WT, Zhu HB, et al. (2012) A nonsense mutation in DHTKD1 causes Charcot-Marie-Tooth disease type 2 in a large Chinese pedigree. Am J Hum Genet 91: 1088-1094.

83. Keeling KM, Wang D, Dai Y, Murugesan S, Chenna B, et al. (2013) Attenuation of nonsense-mediated mRNA decay enhances in vivo nonsense suppression. PLoS One 8: e60478.

84. Establishing a Central Resource of Data from Genome Sequencing Projects: Target Validation Landscape.

85. Noensie EN, Dietz HC (2001) A strategy for disease gene identification through

nonsense-mediated mRNA decay inhibition. Nat Biotechnol 19: 434-439.

86. Zampieri S, Filocamo M, Buratti E, Stroppiano M, Vlahovicek K, et al. (2009) Molecular and functional analysis of the HEXB gene in Italian patients affected with Sandhoff disease: identification of six novel alleles. Neurogenetics 10: 49-58.

87. Linos K, Schwartz J, Kazakov DV, Vanecek T, Carlson JA (2011) Recurrent CYLD nonsense mutation associated with a severe, disfiguring phenotype in an African American family with multiple familial trichoepithelioma. Am J Dermatopathol 33: 640-642.

88. Wilschanski M, Yahav Y, Yaacov Y, Blau H, Bentur L, et al. (2003) Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations. N Engl J Med 349: 1433-1441.

89. Zilberberg A, Lahav L, Rosin-Arbesfeld R (2010) Restoration of APC gene function in colorectal cancer cells by aminoglycoside- and macrolide-induced read-through of premature termination codons. Gut 59: 496-507.

90. Floquet C, Rousset JP, Bidou L (2011) Readthrough of premature termination codons in the adenomatous polyposis coli gene restores its biological activity in human cancer cells. PLoS One 6: e24125.

91. Floquet C, Deforges J, Rousset JP, Bidou L (2011) Rescue of non-sense mutated p53 tumor suppressor gene by aminoglycosides. Nucleic Acids Res 39: 3350-3362.

92. Du L, Jung ME, Damoiseaux R, Completo G, Fike F, et al. (2013) A new series of novel small molecular weight compounds induce readthrough of all three types of nonsense mutations in the ATM gene. Mol Ther.

93. Vecsler M, Ben Zeev B, Nudelman I, Anikster Y, Simon AJ, et al. (2011) Ex vivo treatment with a novel synthetic aminoglycoside NB54 in primary fibroblasts from Rett syndrome patients suppresses MECP2 nonsense mutations. PLoS One 6: e20733.

94. Brumm H, Mahlhaus J, Bolze F, Scherag S, Hinney A, et al. (2012) Rescue of melanocortin 4 receptor (MC4R) nonsense mutations by aminoglycoside-mediated read-through. Obesity (Silver Spring) 20: 1074-1081.

95. Guerin K, Gregory-Evans CY, Hodges MD, Moosajee M, Mackay DS, et al. (2008) Systemic aminoglycoside treatment in rodent models of retinitis pigmentosa. Exp Eye Res 87: 197-207.

96. Goldmann T, Overlack N, Wolfrum U, Nagel-Wolfrum K (2011) PTC124-mediated translational read through of a nonsense mutation causing Usher syndrome type 1C. Hum Gene Ther 22: 537-547.

97. Bellais S, Le Goff C, Dagoneau N, Munnich A, Cormier-Daire V (2010) In vitro read through of termination codons by gentamycin in the StÃ¼ve-Wiedemann Syndrome. Eur J Hum Genet 18: 130-132.

98. Tan L, Narayan SB, Chen J, Meyers GD, Bennett MJ (2011) PTC124 improves read through and increases enzymatic activity of the CPT1A R160X nonsense mutation. J Inherit Metab Dis 34: 443-447.

99. Sanchez-Alcudia R, Perez B, Ugarte M, Desviat LR (2012) Feasibility of nonsense mutation read through as a novel therapeutical approach in propionic acidemia. Hum Mutat 33: 973-980.

100. Perez B, Rodriguez-Pombo P, Ugarte M, Desviat LR (2012) Readthrough strategies for therapeutic suppression of nonsense mutations in inherited metabolic disease. Mol Syndromol 3: 230-236.

101. Harmer SC, Mohal JS, Kemp D, Tinker A (2012) Read through of long-QT syndrome typeÂ 1 nonsense mutations rescues function but alters the biophysical properties of the channel. Biochem J 443: 635-642.

102. Gonzalez-Hilarion S, Beghyn T, Jia J, Debreuck N, Berte G, et al. (2012) Rescue of nonsense mutations by amlexanox in human cells. Orphanet J Rare Dis 7: 58.

103. Amlexanox

104. Aphthasol

105. Bell J (2005) Amlexanox for the treatment of recurrent aphthous ulcers. Clin Drug Investig 25: 555-566.

106. Fu J, Zhu X, Dan H, Zhou Y, Liu C, et al. (2012) Amlexanox is as effective as dexamethasone in topical treatment of erosive oral lichen planus: a short-term pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol 113: 638-643.

107. Elad S, Epstein JB, von Bültzingslöwen I, Drucker S, Tzach R, et al. (2011) Topical immunomodulators for management of oral mucosal conditions, a systematic review; Part II: miscellaneous agents. Expert Opin Emerg Drugs 16: 183-202.

108. Meng W, Dong Y, Liu J, Wang Z, Zhong X, et al. (2009) A clinical evaluation of amlexanox oral adhesive pellicles in the treatment of recurrent aphthous stomatitis and comparison with amlexanox oral tablets: a randomized, placebo controlled, blinded, multicenter clinical trial. Trials 10: 30.

109. Halder SK, Matsunaga H, Ueda H (2012) Neuron-specific non-classical release of prothymosin alpha: a novel neuroprotective damage-associated molecular patterns. J Neurochem 123: 262-275.

110. Nunez-Vergara LJ, Squella JA, Navarrete-Encina PA, Vicente-Garca E, Preciado S, et al. (2011) Chromenopyridines: promising scaffolds for medicinal and biological chemistry. Curr Med Chem 18: 4761-4785.

111. Evdokimov NM, Kireev AS, Yakovenko AA, Antipin MY, Magedov IV, et al. (2007) One-step synthesis of heterocyclic privileged medicinal scaffolds by a multicomponent reaction of malononitrile with aldehydes and thiols. J Org Chem 72: 3443-3453.

112. Thapa U, Thapa P, Karki R, Yun M, Choi JH, et al. (2011) Synthesis of 2,4-diaryl chromenopyridines and evaluation of their topoisomerase I and II inhibitory activity, cytotoxicity, and structure-activity relationship. Eur J Med Chem 46: 3201-3209.

113. Unangst PC, Capiris T, Connor DT, Heffner TG, MacKenzie RG, et al. (1997) Chromeno[3,4-c]pyridin-5-ones: selective human dopamine D4 receptor antagonists as potential antipsychotic agents. J Med Chem 40:2688-2693.

114. Weinstein DS, Gong H, Doweyko AM, Cunningham M, Habte S, et al. (2011) Azaxanthene based selective glucocorticoid receptor modulators: design, synthesis, and pharmacological evaluation of (S)-4-(5-(1-((1,3,4-thiadiazol-2-yl)amino)-2-methyl-1-oxopropan-2-yl)-5H-chromeno[2,3-b]pyridin-2-yl)-2-fluoro-N,N-dimethylbenzamide (BMS-776532) and its methylene homologue (BMS-791826). J Med Chem 54: 7318-7333.

115. Calay ES, Hotamisligil GS (2013) Turning off the inflammatory, but not the metabolic, flames. Nat Med 19:265-267.

116. Koch L (2013) Obesity: Teaching an old drug new tricks--amlexanox targets inflammation to improve metabolic dysfunction. Nat Rev Endocrinol 9: 185.

117. Reilly SM, Chiang SH, Decker SJ, Chang L, Uhm M, et al. (2013) An inhibitor of the protein kinases TBK1 and IKK-Ɛ improves obesity-related metabolic dysfunctions in mice. Nat Med 19: 313-321.

118. Loudon JA, Coleman HG, Allen CM, Schifter M, Ng T, et al. (2012) Rare oral manifestion of chronic myelogenous leukemia and its targeted therapy: A case report and literature review. Uni J Med Dent 1: 79-85

119. Sanchez-Cespedes M, Parrella P, Esteller M, Nomoto S, Trink B, et al. (2002) Inactivation of LKB1/STK11 is a common event in adenocarcinomas of the lung. Cancer Res 62: 3659-3662.

120. Bartolomeo R, Polishchuk EV, Volpi N, Polishchuk RS, Auricchio A (2013) Pharmacological read-through of nonsense ARSB mutations as a potential therapeutic approach for mucopolysaccharidosis VI. J Inherit Metab Dis 36: 363-371.

121. Norman M, Simpson M, Mogensen J, Shaw A, Hughes S, et al. (2005) Novel mutation in desmoplakin causes arrhythmogenic left ventricular cardiomyopathy. Circulation 112: 636-642.

122. Oral & Maxillofacial Pathology. Neville BW, DammDD, Allen CM, Bouquot JE (2008) (3rdedn). WB Saunders Co. Philadelphia, USA.

123. Carulli S, Contin R, De Rosa L, Pellegrini G, De Luca M (2013) The long and winding road that leads to a cure for epidermolysisbullosa. Regen Med 8: 467-481.

124. Groves RW, Liu L, Dopping-Hepenstal PJ, Markus HS, Lovell PA, et al. (2010) A homozygous nonsense mutation within the dystonin gene coding for the coiled-coil domain of the epithelial isoform of BPAG1 underlies a new subtype of autosomal recessive epidermolysisbullosa simplex. J Invest Dermatol 130: 1551-1557.

125. Shaiq PA, Klausegger A, Bauer JW, Azam M, Raja GK, et al. (2012) Compound heterozygous mutations p.Q1530X and 6103delG in COL7A1 causing recessive dystrophic epidermolysisbullosa in a Pakistani family. J Dermatol 39: 472-474.

126. Cuadrado-Corrales N, Sanchez-Jimeno C, Garcia M, Ayuso C, De Lucas R,

et al. (2011) A recurrent nonsense mutation occurring as a de novo event in a patient with recessive dystrophic epidermolysisbullosa. Dermatology 223: 219-221.

127. Ralhan R, Agarwal S, Nath N, Mathur M, Wasylyk B, et al. (2001) Correlation between p53 gene mutations and circulating antibodies in betel- and tobacco-consuming North Indian population. Oral Oncol 37: 243-250.

128. Hassan NM, Tada M, Shindoh M, Hamada J, Kashiwazaki H, et al. (2010) A multiple primary carcinoma consisting of leukoplakia and SCC: a case report with p53 mutation analysis. Anticancer Res 30: 4773-4778.

129. Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, et al. (2012) De novo gene disruptions in children on the autistic spectrum. Neuron 74: 285-299.

130. Tarabeux J, Champagne N, Brustein E, Hamdan FF, Gauthier J, et al. (2010) De novo truncating mutation in Kinesin 17 associated with schizophrenia. Biol Psychiatry 68: 649-656.

131. Kanwar JR, Sriramoju B, Kanwar RK (2012) Neurological disorders and therapeutics targeted to surmount the blood-brain barrier. Int J Nanomedicine 7: 3259-3278.

132. Domínguez A, Alvarez A, Hilario E, Suarez-Merino B, Goñi-de-Cerio F (2013) Central nervous system diseases and the role of the blood-brain barrier in their treatment. Neurosci Discov 1:3.

133. Jagielski T, Grzeszczuk M, Kamiński M, Roeske K, Napiórkowska A, et al. (2013) Identification and analysis of mutations in the katG gene in multidrug-resistant Mycobacterium tuberculosis clinical isolates. Pneumonol Alergol Pol 81): 298-307.

134. Renzoni A, Andrey DO, Jousselin A, Barras C, Monod A, et al. (2011) Whole genome sequencing and complete genetic analysis reveals novel pathways to glycopeptide resistance in Staphylococcus aureus. PLoS One 6: e21577.

135. Totoki Y, Tatsuno K, Yamamoto S, Arai Y, Hosoda F, et al. (2011) High-resolution characterization of a hepatocellular carcinoma genome. Nat Genet 43: 464-469.

136. Lee SA, Kim K, Kim H, Kim BJ (2012) Nucleotide change of codon 182 in the surface gene of hepatitis B virus genotype C leading to truncated surface protein is associated with progression of liver diseases. J Hepatol 56: 63-69.

137. Venkataraman N, Cole AL, Ruchala P, Waring AJ, Lehrer RI, et al. (2009) Reawakening retrocyclins: ancestral human defensins active against HIV-1. PLoS Biol 7: e95.

138. Dontu G, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, et al. (2004) Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. Breast Cancer Res 6: R605-615.

139. Park DM, Jung J, Masjkur J, Makrogkikas S, Ebermann D, et al. (2013) Hes3 regulates cell number in cultures from glioblastomamultiforme with stem cell characteristics. Sci Rep 3: 1095.

140. Ueo T, Imayoshi I, Kobayashi T, Ohtsuka T, Seno H, et al. (2012) The role of Hes genes in intestinal development, homeostasis and tumor formation. Development 139: 1071-1082.

141. Wang NJ, Sanborn Z, Arnett KL, Bayston LJ, Liao W, et al. (2011) Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. Proc Natl Acad Sci U S A 108: 17761-17766.

142. Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, et al. (1996) Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. Nature 383: 707-710.

143. Lin HC, Le Hoang P, Hutchinson A, Chao G, Gerfen J, et al. (2012) Alagille syndrome in a Vietnamese cohort: mutation analysis and assessment of facial features. Am J Med Genet A 158A: 1005-1013.

144. Shiba N, Kanazawa T, Park MJ, Okuno H, Tamura K, et al. (2010) NOTCH1 mutation in a female with myeloid/NK cell precursor acute leukemia. Pediatr Blood Cancer 55: 1406-1409.

145. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, et al. (2011) Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science 333: 1154-1157.

146. Zhang S, Long H, Yang YL, Wang Y, Hsieh D, et al. (2013) Inhibition of CK2Î± down-regulates Notch1 signalling in lung cancer cells. J Cell Mol Med 17: 854-862.

147. Rogers S, Wells R, Rechsteiner M (1986) Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science 234: 364-368.

148. Capaccione KM, Pine SR (2013) The Notch signaling pathway as a mediator of tumor survival. Carcinogenesis 34: 1420-1430.

149. Egloff AM, Grandis JR (2012) Molecular pathways: context-dependent approaches to Notch targeting as cancer therapy. Clin Cancer Res 18: 5188-5195.

150. Bahra M, Kamphues C, Boas-Knoop S, Lippert S, Esendik U, et al. (2012) Combination of hedgehog signaling blockage and chemotherapy leads to tumor reduction in pancreatic adenocarcinomas. Pancreas 41: 222-229.

151. Gangopadhyay S, Nandy A, Hor P, Mukhopadhyay A (2013) Breast cancer stem cells: a novel therapeutic target. Clin Breast Cancer 13: 7-15.

152. Rani SG, Mohan SK, Yu C (2010) Molecular level interactions of S100A13 with amlexanox: inhibitor for formation of the multiprotein complex in the nonclassical pathway of acidic fibroblast growth factor. Biochemistry 49: 2585-2592.

153. Singh S, Singh PP (2013) Statin a day keeps cancer at bay. World J Clin Oncol 4: 43-46.

154. Addou-Klouche L, Adelaide J, Finetti P, Cervera N, Ferrari A, et al. (2010) Loss, mutation and deregulation of L3MBTL4 in breast cancers. Mol Cancer 9: 213.

155. Molofsky AV, He S, Bydon M, Morrison SJ, Pardal R (2005) Bmi-1 promotes neural stem cell self-renewal and neural development but not mouse growth and survival by repressing the p16Ink4a and p19Arf senescence pathways. Genes Dev 19: 1432-1437.

156. Knappskog S, Lonning PE (2012) P53 and its molecular basis to chemoresistance in breast cancer. Expert Opin Ther Targets 16 Suppl 1: S23-30.

157. Horbinski C, Kofler J, Yeaney G, Camelo-Piragua S, Venneti S, et al. (2011) Isocitrate dehydrogenase 1 analysis differentiates gangliogliomas from infiltrative gliomas. Brain Pathol 21: 564-574.

158. Letourneau IJ, Quinn MC, Wang LL, Portelance L, Caceres KY, et al. (2012) Derivation and characterization of matched cell lines from primary and recurrent serous ovarian cancer. BMC Cancer 12: 379.

159. Leftheriotis G, Omarjee L, Le Saux O, Henrion D, Abraham P, et al. (2013) The vascular phenotype in Pseudoxanthomaelasticum and related disorders: contribution of a genetic disease to the understanding of vascular calcification. Front Genet 4: 4.

160. Mungrue IN, Zhao P, Yao Y, Meng H, Rau C, et al. (2011) Abcc6 deficiency causes increased infarct size and apoptosis in a mouse cardiac ischemia-reperfusion model. Arterioscler Thromb Vasc Biol 31: 2806-2812.

161. Loudon JA (2011) A novel prosurvival model for cancer under environmental challenge: the "heart-felt" message for therapeutic intervention. Oncol Res 19: 407-443.

162. Loudon JA (2013) Cancer may lend key clues for cardiovascular risk analysis. Cardiovasc Drugs Ther 27: 255-256.

163. Chen ZS, Tiwari AK (2011) Multidrug resistance proteins (MRPs/ABCCs) in cancer chemotherapy and genetic diseases. FEBS J 278: 3226-3245.

164. Boileau C, Guo DC, Hanna N, Regalado ES, Detaint D, et al. (2012) TGFB2 mutations cause familial thoracic aortic aneurysms and dissections associated with mild systemic features of Marfan syndrome. Nat Genet 44: 916-921.

165. Garg V (2006) Molecular genetics of aortic valve disease. Curr Opin Cardiol 21: 180-184.

166. De Vos WH, Houben F, Hoebe RA, Hennekam R, van Engelen B, et al. (2010) Increased plasticity of the nuclear envelope and hypermobility of telomeres due to the loss of A-type lamins. Biochim Biophys Acta 1800: 448-458.

167. Parks SB, Kushner JD, Nauman D, Burgess D, Ludwigsen S, et al. (2008) Lamin A/C mutation analysis in a cohort of 324 unrelated patients with idiopathic or familial dilated cardiomyopathy. Am Heart J 156: 161-169.