

Lysyl oxidase like-2 (LOXL2)

Chopra, Vriddhi; Sangarappillai, Ruth; Romero-Canelón, Isolda; Jones, Alan M

DOI:

[10.1002/adtp.201900119](https://doi.org/10.1002/adtp.201900119)

License:

None: All rights reserved

Document Version

Peer reviewed version

Citation for published version (Harvard):

Chopra, V, Sangarappillai, R, Romero-Canelón, I & Jones, AM 2020, 'Lysyl oxidase like-2 (LOXL2): an emerging oncology target', *Advanced Therapeutics*, vol. 3, no. 2, 1900119. <https://doi.org/10.1002/adtp.201900119>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

This is the peer reviewed version of the following article: Chopra, V., Sangarappillai, R.M., Romero-Canelón, I. and Jones, A.M. (2020), Lysyl Oxidase Like-2 (LOXL2): An Emerging Oncology Target. *Adv. Therap.*, 3: 1900119., which has been published in final form at <https://doi.org/10.1002/adtp.201900119>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Lysyl Oxidase Like-2 (LOXL2): An Emerging Oncology Target

Vridhhi Chopra,^{a†} Ruth M. Sangarappillai,^{a†} Isolda Romero-Canelón^a and Alan M. Jones^{a*}

^a School of Pharmacy, University of Birmingham, B15 2TT, United Kingdom
E-mail: a.m.jones.2@bham.ac.uk

Keywords: lysyl oxidase like-2, LOXL2, inhibitor, cancer, fibrosis

Abstract

Lysyl oxidase-like 2 (LOXL2) is an emerging drug target for therapy of metastatic disease due to its role in the upregulation of key processes including epithelial-mesenchymal transition (EMT) and invasion. A flurry of recent activity in the field of small molecule LOXL2 inhibitor development by pharmaceutical and academic laboratories has renewed interest in targeting LOXL2.

In this review we: 1) determine the viability of LOXL2 as a drug target for the treatment of metastatic disease through an investigation of its biological actions; 2) consider the pitfalls of an antibody approach; and 3) review the small molecule approaches emerging in the scientific and patent literature. This review identifies that LOXL2 is localised and active intracellularly and extracellularly in invasive cancer cells. LOXL2 has been implicated in a number of established signalling pathways involved in tumour progression, further highlighting its appeal as a target in metastatic disease.

Previously, limited chemical inhibitors have been developed such as β -aminopropionitrile and D-penicillamine however, their selectivity profile for the LOX family has proved controversial. Antibodies, such as simtuzumab, have been developed selectively against LOXL2. Simtuzumab, in particular, progressed into phase II trials but it was ultimately terminated. Small molecule inhibitors of LOXL2 are summarised, some of which are now in the early stages of clinical trials.

Overall LOXL2 is a promising target for the treatment of metastasis in patients with high LOXL2 expression as a personalised medicine, however, the identification of a breakthrough anti-LOXL2 agent is eagerly awaited.

1. Introduction

1.1. Tumour Metastasis

Advances in cancer therapy and detection through the use of screening tools have contributed to survival rates doubling in the last 40 years.^[1] Nonetheless, it is estimated that metastatic disease is responsible for 90% of all cancer deaths; a number which has had negligible change in the past decades. Even with the use of systemic therapy, dynamic regulation of signalling pathways involved in the progression of cancer, changes in cellular phenotype, and therefore the development of resistant cancer cells, has made cancer metastasis difficult to target.^[2] Understanding the underlying processes by which metastasis occurs is, therefore, key to drug discovery and improving clinical outcomes.

Metastatic cancer involves a development stage in which cancer cells from the primary tumour growth acquire a migratory phenotype and spread to distant organs *via* the bloodstream. The cancer growth at the secondary site carries the same characteristics of the initial tumour. At this point, treatment becomes more challenging as treatment options are limited and prognosis worsens.

A retrospective histological analysis of post-mortem autopsies^[3] ($n = 3,827$; between 1913-1943) established patterns in metastasis over a broad range of cancers in patients who had not received chemotherapy. The results showed that, after lymph nodes, liver, lung and bone were the most common sites of secondary tumour growth. Furthermore, although the discovery of new cancer therapies has reduced the rate of progression, patterns of migration have not changed; liver, lung and bone remain the most common sites of metastasis.^[4]

Cancers may also metastasize to the brain, which can result in increased intracranial pressure, leading to cognitive decline or motor neurone dysfunction.^[4] The treatment for such metastases is further complicated as the blood-brain barrier (BBB) limits the types of agents suitable.^[5] Given the potential

for metastatic cancer to impair quality of life and increase mortality, it is imperative to understand the mechanisms involved in migration to identify viable drug targets.

1.2. Cellular Migration

In order for cancer cells to metastasize, it is widely accepted that cells adopt motile characteristics *via* the phenomenon known as epithelial-mesenchymal transition (EMT).^[6] EMT results in epithelial cells losing their cell-cell adhesions and cell polarity, resulting in invasive and migratory functionality associated with the mesenchymal phenotype. This process results in the loss of epithelial protein expression, namely E-cadherin, which stabilizes cells *via* adhesion complexes and promotes the expression of mesenchymal proteins, exhibiting a migratory phenotype.^{[7][8]} This transformation enables epithelial cells within tumours to turn into cells that can invade both nearby and distant tissues. EMT can be induced by growth factors and extracellular matrix (ECM) constituents. The progression of EMT is controlled by a network of signalling and transcription factors. Various transcription factors have been identified as EMT-inducers, however SNAIL, ZEB and TWIST are the most extensively studied and proposed to be the principal inducers.^{[7][9]} Each of these transcription factors play a pivotal role in modifying or repressing the *CDH1* promoter, which is responsible for the coding of E-cadherin, consequently altering the epithelial phenotype.^[7] In the simplest scenario, once a migratory phenotype is established, the aggressive cancer cells must overcome further steps for successful metastasis; including invasion and migration, intravasation, circulation, extravasation and colonization (**Figure 1**). In the final sequence the metastatic cells must colonize the secondary location *via* cell proliferation. This step is termed the mesenchymal-epithelial transition (MET); the reverse of EMT.^{[10][11][12][13]}

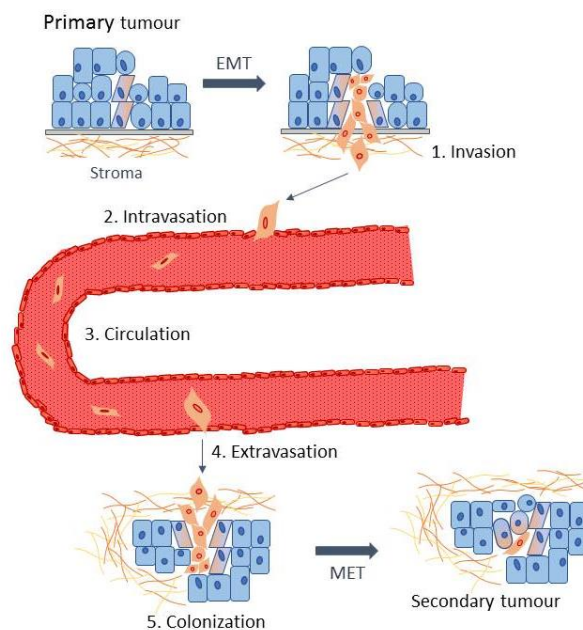


Figure 1. A simplified metastatic cascade from a primary tumour.

Each step is crucial for metastasis to occur, however, due to the complexity of the process and the number of proteins and signalling molecules involved, targeting metastasis has proven challenging. The initial steps, EMT and invasion are ideal for drug therapeutic strategies as this will halt the metastatic cascade early on. Transcription factors and re-arrangements of myofilaments are known to be involved in EMT and invasion and are regulated *via* changes in the stromal environment.^[14] Such processes are stimulated by other factors including activation of the TGF β receptor, which induces EMT transcription factors, or the Wnt signalling pathway which disrupts cellular junctions thus enabling the cell to break free from the tumour mass.^{[15][16]}

The focus of this review, the endogenous enzyme, Lysyl oxidase-like 2 (LOXL2), has emerged as a potential regulator of metastasis. LOXL2 is an upstream regulator of EMT, invasion and a multitude

of metastatic pathways. Targeted selective inhibition or modulation of LOXL2 may be the missing link in the fight against cancer. LOXL2 also holds the potential to be a key stratification predictor in patients for personalised oncology medicine.

2. Lysyl Oxidase-Like 2 (LOXL2)

The Lysyl oxidase (LOX) family are a family of copper-dependent amine oxidases comprised of five members; LOX and LOX-like 1-4 (LOXL1-4), which are divided into two subfamilies based on their structures (**Figure 2**).^{[17][18]} All LOX enzymes have a highly conserved carboxyl-terminal containing the copper binding motif, a lysine tyrosylquinone cofactor (LTQ) and a cytokine receptor-like domain (CRL); essential for its normal catalytic activity. These enzymes are further subcategorised based on their structure particularly at the *N*-terminal, as this differs slightly between the enzymes. LOX and LOXL1 are in one group as they have pro-sequences, which are cleaved to activate the enzymes. LOX and LOXL1 are secreted enzymes and activated outside the cell.^{[19][20]} LOX and LOXL1 have a basic propeptide sequence additionally, LOXL1 has a proline rich domain in the *N*-terminus. However LOXL2-4 have four scavenger receptor cysteine-rich (SRCR) domains; it is postulated that this SRCR domain is involved in protein-protein interactions (PPI) of soluble and membrane-bound protein receptors. LOXL2 is the most extensively studied member of the family and may be a suitable potential target for cancer therapy. These SRCR domains within LOXL2 are thought to be involved in the interactions between proteins in the extracellular matrix (ECM) and cell-surface proteins.^{[19][21][22]} The catalytic domain of LOXL2 is 88% and 86% similar to LOXL3 and LOXL4, respectively.^[23] However for LOX and LOXL1, LOXL2 is 68% and 66% similar, respectively.^[23]

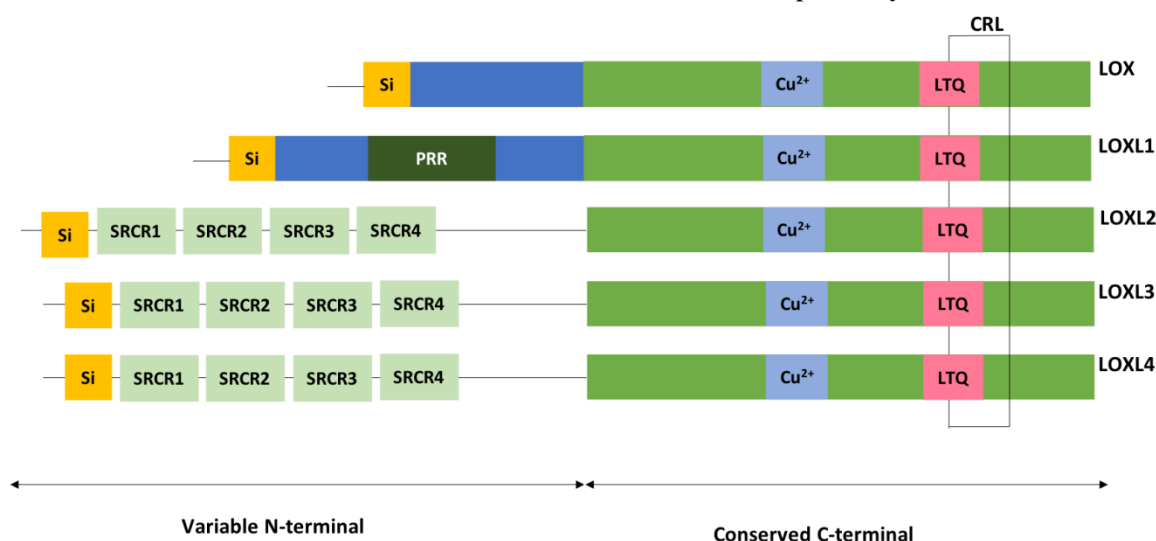


Figure 2. Structures of the enzymes in the LOX family^[21]

Despite the important proposed mechanism of action of LOXL2, the lack of a solved crystal structure of its functionally activated form continues to limit the identification of selective small molecule inhibitors.^[23] In 2018, the crystal structure of human LOXL2 (*h*LOXL2) (**Figure 3**) in its precursor state without the LTQ cofactor was reported. Importantly, the copper binding, histidine rich, site of *h*LOXL2 is occupied by Zinc²⁺ which prevents LTQ generation. The crystal structure of LOXL2 in its functional state (with LTQ cofactor) has to date eluded solution.^[22] This precursor crystal structure of LOXL2 has however assisted in generating a 3D model for the LOX family.^{[24][25]}

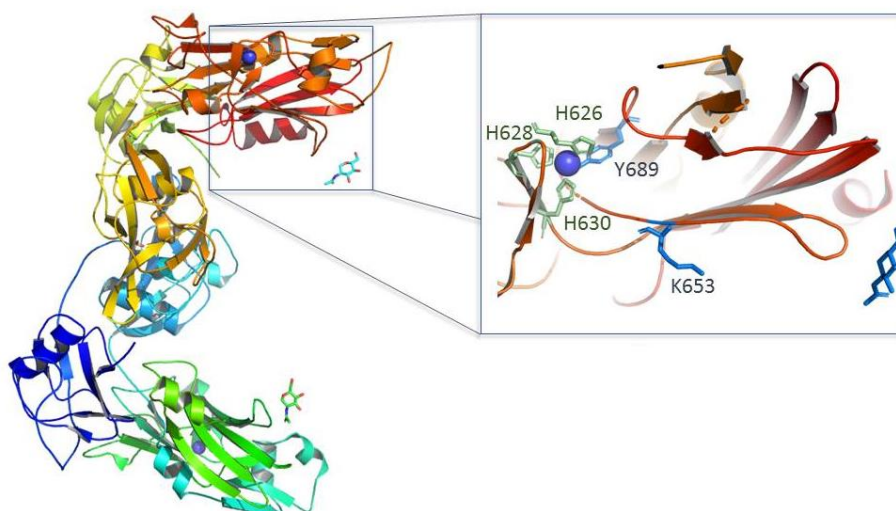
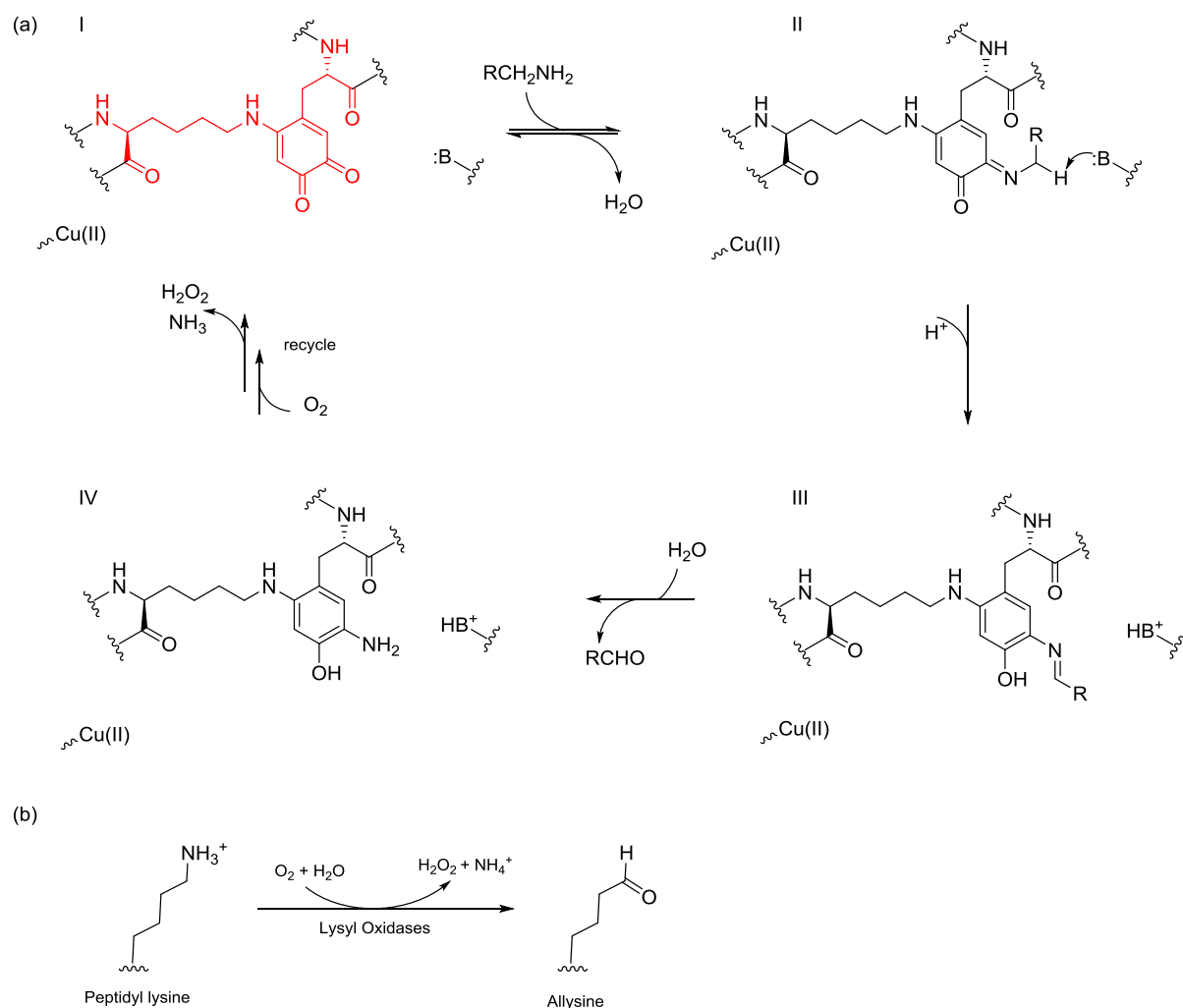


Figure 3. Crystal structure of the precursor form of LOXL2 and inset the amino acid motifs that locate the catalytic domain in the functional state. The LTQ region has been localised to the residues Y689 and K653. The blue sphere represents Zn^{2+} which interacts with H626, H628 and H630 preventing Cu^{2+} from binding and the formation of the LTQ cofactor (PDB: 5ZE3).^[22]

The LTQ region in LOXL2 is defined by the residues Y689 and K653 (**Figure 3**).^[22] The primary role of LOX enzymes is to catalyse the oxidative deamination of lysine and hydroxylysine residues in collagen and elastin of the extracellular matrix (ECM). As a consequence, the reactive aldehyde, allysine is formed which can create *intermolecular* and *intramolecular* covalent bonds with other lysine or allysine residues, cross-linking the ECM. This is an important role in the formation and repair of the ECM network and the construction of connecting tissues.^{[22][26]}



Scheme 1. (a) Mechanism for the oxidation of primary amines. The structure of the LTQ cofactor is highlighted in red; and (b) simplified overall transformation.

The mechanism, by which LOXL2 carries out its endogenous role, involves four main steps (**Scheme 1**). Firstly, the side chain amine from lysine residues in collagen (or elastin) of the ECM reversibly binds to a carbonyl group of the LTQ domain forming a Schiff base. Secondly, a proton is abstracted by a basic residue in the LOXL2 structure, proximal to the LTQ cofactor. This proton abstraction is driven by the formation of a stable aromatic ring within the LTQ region. Thirdly, the arylimine is hydrolysed, releasing the aldehyde (allysine) and producing an aminophenol. Fourthly, steps involving O_2 and Cu^{2+} regenerates the LTQ cofactor to catalyse further cross-linking. Ammonia (NH_3) and hydrogen peroxide (H_2O_2) are released as by-products in this process.^{[27][28]}

If this process is dysregulated, it can result in an excessive level of cross-linking thus stiffening the ECM; underlying the chemical process by which fibrosis occurs. Furthermore, each cycle of the reaction yields H_2O_2 which has been shown to activate signalling pathways known to be involved in tumour progression.^{[29][19]} Immunohistochemistry of human biopsies show that LOXL2 expression is elevated in neoplastic cells compared to normal cells.^[30]

It remains unclear whether LOXL2 acts intracellularly, extracellularly or both in cancer metastasis. As a result, the mechanism of action of its role in cancer progression is poorly understood and the exact mechanism has not been clearly defined thus far.^[31] The role of LOXL2 in ECM collagen crosslinking, mediated by the aforementioned deamination of lysine residues (**Scheme 1**), has subtle peculiarities. Firstly, LOXL2 has been shown to be processed extracellularly by serine proteases at

K317 generating a processed 65 kDa form lacking the first two SRCR domains. It has been shown that full length LOXL2 is active *in vitro* with soluble collagen substrates but inactive to crosslink insoluble collagen within the ECM.^[32]

Secondly, this post-translational modification of LOXL2 at K317, has been shown to be processed in a similar manner in ductal carcinoma patient tissues *via* the proprotein convertase, PACE4. This difference in LOXL2 processing compared to LOX (which requires cleavage of the propeptide for catalytic activation) was found to be non-essential for tropoelastin oxidation or collagen type IV crosslinking *in vitro*. Yet, LOXL2 processing enhances collagen crosslinking by approximately 2-fold, this key difference in LOXL2 processing versus LOX, may enable the identification of unique functions of LOXL2 isoforms in cancer progression.^[33]

2.1. LOXL2 role in EMT and invasion

LOXL2 expression has been identified to promote breast and gastric cancer metastasis.^[34] In the most studied, MCF-7 breast cancer cell line, expressing wt-LOXL2 exhibited increased invasiveness *in vivo* mouse model and induced EMT in these cells.^[35] The role of LOXL2 in invasion and metastasis has been extensively described elsewhere.^{[36][37][38]} In addition to its extracellular activity, LOXL2 has been reported to have an intracellular role in carcinoma cells (**Figure 4**). Intracellular LOXL2 prevents SNAIL degradation (involved in the EMT phenotype), thereby enhancing its expression and consequently represses *CDH1*.^[39] Furthermore, LOXL2 has also been implicated in catalysing the deamination of trimethylated lysine 4 of histone 3 (H3K4); a modification linked to active transcription.^{[7][41][42]} The mechanism by which H3K4 deamination occurs is poorly understood, however it is reported that LOXL2 releases the trimethylated amino group, converting lysine 4 (K4) to the aldehyde, allysine.^{[42][43]} This interaction also represses the activity of *CDH1*, providing a SNAIL-independent pathway in the induction of EMT.^[41]

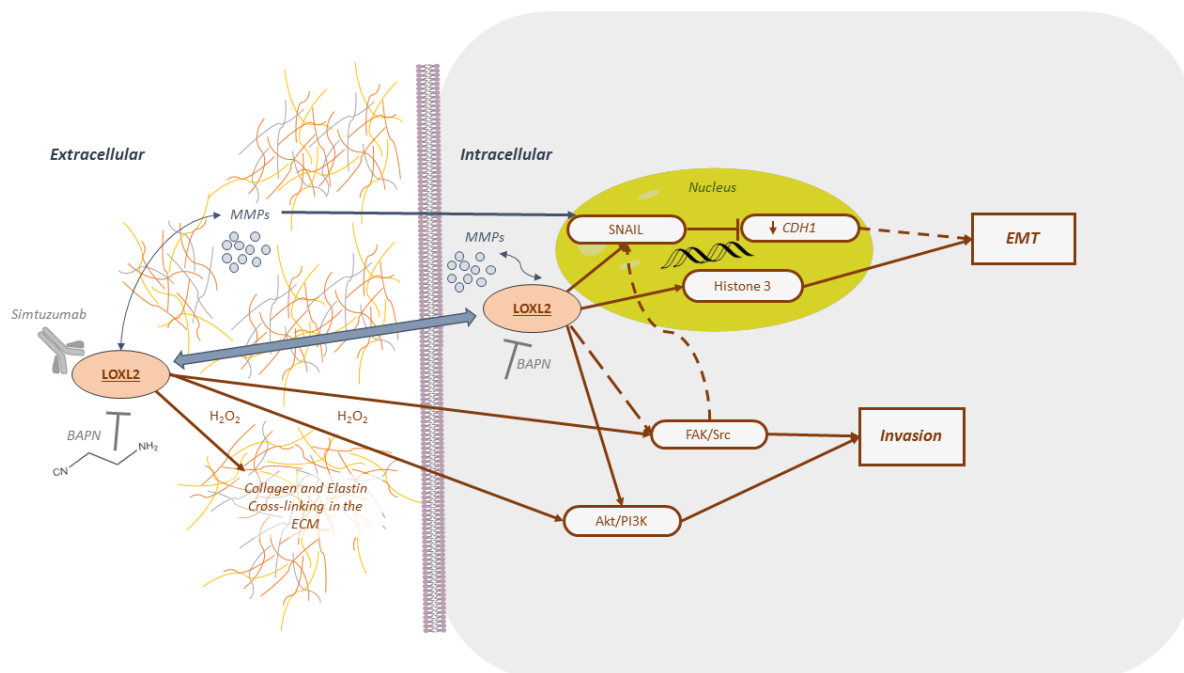


Figure 4. Proposed intracellular and extracellular signalling pathways of LOXL2 in cancer metastasis.

LOXL2 has also been associated with gastric cancer metastasis; H_2O_2 production stimulates the upregulation of Src phosphorylation in the Src Kinase/ Focal adhesion kinase pathway (FAK/Src).^[44] FAK is an intracellular tyrosine kinase, widely accepted as a promoter of cell mobility through the assembly and disassembly of cellular adhesions. Activated FAK recruits Src to the focal adhesion

sites at the leading edge of the migrating cells, forming a complex which then phosphorylates other proteins important in the migratory process.^[45]

FAK/Src signalling has been shown in breast cancer models to be an upstream signalling component of phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathway which has been linked to cell proliferation, tumorigenicity and cellular motility. Furthermore, there have been reports that H₂O₂ produced by LOXL2 enzymatic activity, stimulates the PI3K/Akt pathway, independent of FAK/Src signalling pathway, indicating the many important roles of LOXL2 in cancer.^[19]

2.2. LOXL2 role in fibrosis

An increasing number of studies indicate that LOX enzymes are linked to diseases such as fibrosis. The result of fibrosis is excessive deposition of collagen. This can subsequently lead to organ failure due to the significant scarring of healthy tissue in this disease state.^[20] Several studies have highlighted the link between high levels of LOXL2 and fibrosis.^{[46][47]} For instance, elevated levels of LOXL2 are seen in renal fibrosis.^[48] Likewise, increased levels of LOXL2 have been identified in the epithelium and connective tissues of overgrown gingivitis compared to the tissue with the absence of this disease.^[49]

Currently, there are limited treatment options for fibrosis that have moderate benefits.^[20] LOXL2 has been studied in human lung sections of healthy and IPF donors.^[50] Within the LOX family both LOX and LOXL2 were highly expressed in fibrotic conditions.^[50] Moreover, LOXL2 had a greater collagen oxidation capacity than LOXL3, contradictory to the hypothesis that LOXL3 had a higher oxidation capacity.^[50] When the expression pattern of LOXL2 in IPF was observed, it was found that LOXL2 had an important role in the activation of fibroblasts and contained a high crosslinking capacity.^[50] It was found that in lung fibroblasts, enzymatic activity is needed for LOXL2 mediated modification in the ECM, which changes the α -SMA production in fibrotic conditions.^[50] There was also a reduction of α -SMA and collagen I α when LOXL2 expression was decreased.^[51] These results showed that LOXL2 has the greatest role in fibrotic remodelling within the LOX family.^[50] Antibodies against LOXL2 have been tested in IPF^[41] and small molecule inhibitors against LOXL2 are under development to treat fibrotic diseases including IPF.^[41] LOXL2 may be more prominent in hepatic fibrosis in comparison to the LOX family and is key in the stabilisation of the fibrotic matrix of the liver.^[46] Inhibiting LOXL2 reduced the level of highly crosslinked collagen deposited in liver fibrosis.^[46] As LOXL2 is prominently involved in fibrotic diseases, it is an attractive therapeutic target for this disease.^[47]

3. Is LOXL2 a viable cancer target?

LOXL2 has been studied and implicated in a number of different cancer lineages, exhibiting variation between the reported signalling pathways involved and the localization of LOXL2 in each subtype of cancer.

The role of LOXL2 has been most extensively studied in breast cancer models *in vitro* and *in vivo*. However, phase II clinical trials with the antibody, Simtuzumab have been approved for patients with metastatic pancreatic cancer and KRAS mutant colorectal cancer.

3.1. Antibody approaches leading to Simtuzumab

Antibodies have been developed as an approach to inhibit LOXL2. The murine antibody, AB0023 (GS-607601)^[52] has shown to have a broad application in the field of oncology and fibrosis. Furthermore, it has been tested in cells and mice models.^[30] Simtuzumab (AB0024/GS-6624) is the humanised monoclonal antibody version of AB0023 which has been tested in human participants.^[30] Simtuzumab is an IgG4 monoclonal antibody, which non-competitively inhibits extracellular LOXL2 *via* allosteric inhibition; binding to the fourth SRCR domain (**Figure 4**).^{[20][30]}

AB0023, has been tested in cell lines and mice models before the humanised version entered clinical testing. AB0023 has been studied in the context of LOXL2 involvement in interstitial fibrosis and heart failure in mice dosed at 30 mg/kg twice a week.^[53] The transaortic constriction dysfunction was less seen in the treated mice and the transaortic constriction stressed heart also stabilised left

ventricular fractional shortening.^[53] It was concluded that LOXL2 has an effect on interstitial fibrosis and the dysfunction of the heart as they are involved in the activation of myofibroblasts, promoting the production of collagen within cardiac tissue.^[53]

AB0023 has also been tested in a rabbit model with glaucoma trabeculectomy.^[52] The LOXL2 antibody was compared against a LOX antibody (M64). It was shown post-surgery that LOX and LOXL2 were increased in the Tenon's capsule and conjunctiva of the eye.^[52] Both antibodies decreased fibrosis however, the LOXL2 antibody further decreased blood vessel density and inflammation.^[52] The LOXL2 antibody modulated wound healing properties, which assisted the prognosis of the eyes post-surgery in this study.

Similarly, AB0023 was tested in C57BI/6J mice and decreased the level of crosslinked collagen.^[45] Treatment with the antibody had a greater effect in the later stages of liver fibrosis.^[45] It was shown that the antibody was able to reverse hepatic fibrosis, induced by thioacetamide. Therefore, blocking LOXL2 in the ECM is promising for the treatment of fibrosis.^[45]

AB0023 (15 mg/kg) has been used in SKOV-3 tumour breaking mice which express LOXL2.^[54] Using the antibody alone was insufficient to produce a statistically significant reduction of the tumour volume.^[54] However, using this in combination with Taxol (5 mg/kg) showed decreased tumour volume more significantly.^[54] It was thought that these effects were due to the antibody normalising the structure of the vasculature, improving the delivery of Taxol to the tumour.^[54] Blood-marrow-derived cells, important in the angiogenesis in tumours, were inhibited with AB0023.^[54] This highlighted that the use of a LOXL2 antibody can be beneficial in angiogenic diseases and can improve the delivery of chemotherapeutic options.^[54]

Furthermore, AB0023 has been effective in xenograft models of both primary and metastatic cancers.^[30] AB0023 showed positive results in fibrosis models such as the liver and the lung. The mice and humanised variant of the AB0023 performed well in safety studies and was well tolerated, in mice, rats and cynomolgus monkeys.^[30] The antibody was used to treat tumours from the MDA-MB-435 cell line in mice, and showed to reduce tumour volume significantly which was not seen with the M64 antibody.^[30] The antibody against LOXL2 also decreased the activation of the growth factor, TGF- β 1, which promotes fibroblast activation.^[30] Furthermore, AB0023 was found to be superior to a small molecule, BAPN.^[30]

Intriguingly, an affinity-purified rabbit anti-human LOXL2 polyclonal antibody against the C-terminal of LOXL2 was tested against BGC823 cells.^[44] The treatment decreased the growth and metastasis of these cancer cells in a dose dependent manner.^[44] This trend was also witnessed in a human xenograft model using the BGC823 cell line. LOXL2 is therefore an important target in gastric cancer metastatic treatment.^[44]

Using a 4T1 syngeneic model of breast cancer metastasis treated with an LOXL2-antibody resulted in a lower number of metastases in the lung. This supports the development of an antibody approach in the treatment of cancers, particular metastatic breast cancer.^[55] Additionally, using an LOXL2-antibody in 4T1 mouse breast cancer cells decreased the expression level of α -SMA, implying that fibroblast activation of these cells was reduced following treatment.^[56]

Simtuzumab has been reached clinical trials for the treatment of fibrosis in addition to metastatic cancer. A summary of the most advanced (phase II) clinical trials data for Simtuzumab is shown in **Table 1**.

Table 1. Clinical phase II trials for Simtuzumab.

| Entry | Disease | Doses | Main outcomes | Adverse effects | Additional comments | References |
|-------|--|--|---|---|---|------------|
| 1 | Pancreatic adenocarcinoma | 1000 mg/m ² gemcitabine with 200 mg or 700 mg simtuzumab or placebo. | Not effective in progression-free survival but well tolerated. | Frequency of adverse events was similar in both groups. | A multicentre, randomised, double-blinded, placebo-controlled phase II trial (n = 240). | [57] |
| 2 | Second-Line treatment of metastatic <i>KRAS</i> mutant colorectal adenocarcinoma | Simtuzumab 200 mg or 700 mg FOLFIRI (Folinic acid 200 mg/m ² , 5-FU 2400 mg/m ² , and Irinotecan 180 mg/m ²) | The addition of Simtuzumab to FOLFIRI did not improve clinical outcomes in patients with metastatic <i>KRAS</i> mutant CRC. | The adverse events were reported to be similar between treatment and control groups. There were 7 deaths in the group, and one ascribed to FOLFIRI usage. | A dose ranging, randomised, double-blinded, placebo-controlled phase II trial (n = 249). | [58] |
| 3 | Idiopathic pulmonary fibrosis | Pirfenidone and nintedanib with either 125 mg/mL simtuzumab or placebo. | Simtuzumab is not supported in the use of IPF. | Similar side effects were reported between the groups. | This was a randomised, double-blinded phase II trial (n = 544). | [59] |
| 4 | Primary Sclerosing Cholangitis | Simtuzumab 75 mg, 125 mg or placebo. | No clinical benefit for patients with primary sclerosing cholangitis. | Four patients had adverse effects related to the treatment and therefore treatment was stopped in these patients. | A dose-ranging, randomised, double-blinded, placebo-controlled phase IIb trial (n = 234). | [60] |

| | | | | | | |
|---|---|--|--|--|---|--------------|
| 5 | Primary myelofibrosis (MF), post-polycythaemia vera MF and post-essential thrombocythaemia MF | Simtuzumab 200 mg or 700 mg alone or with ruxolitinib. | Both groups were well tolerated but no clinical benefit in either groups. It did not reduce bone marrow fibrosis in MF. | Two deaths within the study related to disease progression rather than the treatment. | A randomised, open-label phase II trial (n = 54). | [61] |
| 6 | Advanced fibrosis caused by non-alcoholic steatohepatitis | Simtuzumab 75 mg or 125 mg or placebo. | A significant reduction in the content of hepatic collagen but there was not a significant difference between the groups that received 75 mg or 125 mg simtuzumab and placebo. Due to this reason the trial was stopped. | Adverse events were reported to be similar between treatment and control groups. There was one death in both 75 mg group and placebo group. | A dose-ranging, randomised, double-blinded, placebo-controlled phase IIb trial (n = 219). | [62][63][64] |
| 7 | Compensated cirrhosis | Simtuzumab 200 mg or 700 mg or placebo. | Treatment was not effective in reducing the hepatic venous pressure gradient. Therefore, this trial was stopped. | The adverse events were reported to be similar between treatment and control groups. There were two deaths in the 700 mg group and one death in the placebo group. | A dose-ranging, randomised, double-blinded, placebo-controlled phase IIb trial (n = 258). | [62][63][65] |

Phase II clinical trials were conducted with the humanized IgG monoclonal antibody, Simtuzumab in conjunction with Gemcitabine in patients with metastatic PaCa (**Table 1**, entry 1). Eligible patients were recently diagnosed with metastatic PaCa and were assigned 1,000 mg/m² gemcitabine with either placebo, 200 mg or 700 mg simtuzumab at a 1:1:1 ratio. A total of 240 patients were recruited in this double-blind randomised control trial.^[57] Patients received the treatments on days 1, 8 and 15 of each 28-day cycle, with the primary end-point being progression-free survival. Phase I clinical trials demonstrated safety, however there was no improvement in clinical outcomes for patients participating in the phase II trial.^{[66][57]} It is noteworthy that Gemcitabine did not have a role on the expression of LOXL2 in pancreatic carcinoma.^[67]

There are a number of reasons as to why this clinical trial may have failed; the first of which is that, in the phase I open label study, 1 patient out of 32 had pancreatic cancer.^{[66][68]} Although tumour size had decreased by 56% in this patient, this type of tumour was a pancreatic neuroendocrine tumour which only accounts for 5% of all pancreatic tumours and is phenotypically different to the patients enrolled in the phase II trial who had PaCa.^[66]

Secondly, Simtuzumab was not efficacious in this particular cohort group because it is a non-competitive allosteric inhibitor of LOXL2 which acts extracellularly.^[69] Despite immunohistochemistry results showing that pancreatic adenocarcinoma has high expression of LOXL2 in the stromal environment, Herranz^[42] demonstrated that LOXL2 is also localized within MIA-PaCa2 cells. This could therefore mean that the effects of Simtuzumab are sub-therapeutic due to the intracellular and extracellular localisations of LOXL2 in these cancer cells. Finally, due to the erratic expression of LOXL2 in various cancer cell lines with no direct correlation to migratory potential,^[70] LOXL2 may be in fact a poor therapeutic target. Despite the failure of the clinical trial, a positive outcome was that the safety profile of the placebo group compared to the groups treated with Simtuzumab. Simtuzumab therefore may be suitable for use for other conditions such as fibrosis in future studies.

Phase II clinical trials were approved for Simtuzumab in patients with KRAS mutant colorectal cancer. KRAS mutations are prevalent in 30-40% of colorectal cancer cases and is associated with poorer survival rates than wild-type KRAS.^[71] The trial (**Table 1**, entry 2) was conducted in a similar manner as the phase II clinical trials with metastatic PaCa patients; a double-blind randomised control trial with three arms.^{[57][58]} In this trial, 249 patients were enrolled, and all received FOLFIRI (Folinic acid, Fluorouracil and Irinotecan) with either placebo, 200 mg or 700 mg of simtuzumab in a 1:1:1 ratio on days 1 and 15 of each 28-day cycle.^[58]

Simtuzumab exhibited a good safety profile compared to the placebo group.^[58] However, despite greater supporting evidence in prior cellular studies in both the phase I & Iia clinical trials, Simtuzumab failed to show improved clinical outcomes when compared with the control arm.^{[66][58]} Cellular and human tissue studies conducted by Park^[72] showed a strong association between LOXL2 and metastatic potential, therefore it was surprising that the clinical trial showed no effect in this patient group. The failure of the clinical trial may again be attributed to the fact that Simtuzumab is limited to targeting extracellular LOXL2, suggesting that LOXL2 acts *via* intracellular mechanisms in cancer metastasis.

Simtuzumab has failed in multiple phase II trials in both fibrotic and cancerous conditions (**Table 1**) bringing into question whether LOXL2 is a viable drug target for the treatment of metastatic disease.^{[73][74]}

3.1.1. Antibody GS341

An alternative antibody that targeted the active site of LOXL2, is referred to as GS341.^[75] This antibody inhibited crosslinking of collagen which leads to the disorganisation of these collagen fibres. GS341 was compared against GS021 (which is an inactive antibody clone), and showed a stronger effect.^[75] It was predicted that GS341 bound to the active site of the enzyme as it did not have an effect in the presence of elastin (a substrate of LOXL2).^[75] GS341 tested in MDA-MB-231 cells decreased the invasive nature of this cell line in a dose-dependent manner.^[75] This effect was different

to another antibody, GS092, which did not have an impact on the invasive nature of this cell line, highlighting that the activity of the enzyme is important for the cancerous nature of this breast cancer cell line. It was further identified that LOXL2 mediated collagen fibril alignment which controls tumour associated cell processes, directed by collagen fibres orientation.^[75]

3.1.2.Summary of the antibody approach

The use of a variety of LOXL2 antibody treatments in preclinical models, especially AB0023 as an allosteric mouse monoclonal antibody in xenografted tumour models, led to a decrease in metastatic behaviour in some systems. These studies, together with loss of function and genetically modified models support a role for LOXL2 in tumour progression and metastasis.

Yet, clinical trials with the humanized version of AB0023 antibody (Simtuzumab) have to date, failed in pancreatic, colorectal and breast carcinomas (and fibrotic diseases). Therefore, the antibody approach is not an effective therapy for human tumours (or fibrotic diseases). Do the underlying mechanisms of LOXL2 in carcinoma's bring clarity to this failure? We next consider the role of LOXL2 in a range of cancers.

3.2.Breast Cancer

The role of LOXL2 in cancer metastasis has been most extensively studied in breast cancer models.^{[17][55][36]} A retrospective study of 295 breast cancer patients showed a positive correlation between LOXL2 expression, metastases and significantly poorer survival rates. A prospective study of 309 patients found that 16.2% of patients expressed LOXL2.^[76] Patients with triple negative breast cancer (TNBC) had a statistically significant 12.3% higher rate of LOXL2-positive expression, than non-TNBC.^[76] Furthermore, LOXL2-mediated oxidation of histone H3K4 is also higher in TNBC, and knock down experiment on LOXL2 resulted in reduced H3K4ox levels and sustained activation of the DNA damage response.^[77]

These findings are supported by the identification of LOXL2 upregulation in invasive metastatic breast cancer models, Hs578T and MDA-MB231, in comparison to poorly invasive breast cancers, MCF-7 and T47D.^[51] RT-PCR analysis demonstrated LOXL2 mRNA expression to be upregulated in the cell lines, Hs578T and MDA-MB231 however not expressed in MCF-7 and T47D cell lines.^[51] This suggests that LOXL2 expression is associated with an invasive phenotype. These results are supported by ectopic overexpression of LOXL2 in MCF-7 cells resulting in an invasive phenotype.^[35] Migratory assays showed that silencing LOXL2 significantly reduced the number of invasive cells.^{[78][76]} The percentage of migration showed a statistically significant decrease in migration of both cell types, BT549 and MDA-MB-231, when LOXL2 expression was silenced.

Expression and effects of LOXL2 are cell-specific which suggests anti-LOXL2 therapy would be more suitable for personalised medicine for a sub-group of cancer patients which are LOXL2-positive. However, in order to target LOXL2 effectively, pinpointing the site of action in cancer cells is fundamental in developing an anti-LOXL2 compound.

Immunohistochemical analysis of normal breast tissue and breast cancer tissue determined the localisation of LOXL2 changes in the disease state.^[35] These studies confirmed that in breast cancer tissues, LOXL2 is localised in the cytoplasm, whereas LOXL2 is localised around the membrane in normal tissue. These findings were consistent with lung cancer tissue and normal lung tissue, thereby supporting the idea that LOXL2 acts intracellularly in cancer tissue.^[79]

There is also an emerging role of secreted LOXL2 which has been reported to be an inducer of invasion in breast cancer,^{[17][51]} with studies of the effect of the extracellular environment in LOXL2 expression of poorly invasive breast cancer cells, MCF-7. The results showed that MCF-7 cells had no mRNA expression of LOXL2, however, when grown in a fibroblast conditioned matrix, LOXL2 expression was upregulated.

Moreover, the fibroblast cells also expressed LOXL2, however, LOXL2 mRNA was not present in the conditioned matrix. Therefore, the upregulation of LOXL2 in MCF-7 in the conditioned matrix

cannot be due to DNA or RNA transfer from the fibroblasts which conditioned the matrix.^[51] The implication being the tumour microenvironment is stimulating the upregulation of LOXL2. In addition to cellular studies and human tissue analysis, *in vivo* breast cancer models have also been used to analyse the correlation between inhibiting LOXL2 expression and metastasis to local or distant sites.

Transfected mice with MDA-MB-231 cell lines alongside a LOXL2 silencer (shLOXL2),^[55] when compared to the control, untreated mice, shLOXL2 mice had significantly fewer lung metastases. The same study with 4T1 cells, which readily metastasize to the liver and bone also showed a significant decrease in metastatic disease within these organs.^[55] It was also observed, that the metastatic masses at the secondary sites in the lung and liver, were much smaller in the shLOXL2 mice groups than in the control. Providing further evidence for the role of LOXL2 in metastasis.^[55]

Recently, PyMT mouse models with either deleted or over-expressed LOXL2 in the mammary glands of these mice; expression was confirmed by mRNA analysis.^[38] These transgenic mouse models are typically characterised to have <85% rate of metastasis to the lung and lymph node at 3.5 months.^[80] After 16 weeks, the metastasis incidence was examined; the results revealed that there was a 75% decreased rate of metastasis in the LOXL2 knockout mice in comparison to control mice. Additionally, mice that overexpressed LOXL2 demonstrated an increase in metastatic incidence to the lungs. This was further supported by circulating tumour cell (CTC) analysis, which showed a positive correlation between LOXL2 expression and CTCs. The knockout mice to have the least amount of CTC followed by the control, and then the LOXL2 over-expressing mice, which had the greatest number of CTC, indicating a high potential for metastases.^{[81][38]} These results indicate that LOXL2 plays a vital role in metastasis to the lung in this model, in agreement with earlier findings.^[55]

Multiple reports have shown that MMP's and LOXL2 interplay to facilitate invasion.^{[55][82][83]} LOXL2 inhibition resulted in a decrease in TIMP1, a subtype of MMP.^[55] This decrease in TIMP1 was shown to be related to tumour invasiveness when compared with silencing LOXL2 alone and then adding recombinant human TIMP1 in MDA-MB-231 cells.

Silencing of LOXL2 significantly decreases the number of invading tumour cells, the addition of recombinant TIMP-1 reverses this effect, which was also found to be significant. Visual inspection of these cells in 3D Matrigel, with the same interventions, also showed a similar pattern. This suggests that the role of LOXL2 is important in facilitating invasion *via* its extracellular function by upregulating MMP production and thereby creating pockets for cells to migrate out of, in addition to regulating EMT *via* its aberrant intracellular functions; highlighting the importance of the stromal environment in tumour metastasis.

This is however contradicted in the findings of *in vivo* studies^[38] that examined the metastatic niche. They found no significant difference in biomechanical properties in the ECM of control and treated mice. This may be attributed to the different methods used^[38] this study assessed changes in stiffness rather than the expression of ECM components.

3.3. Pancreatic Cancer

Pancreatic cancer has the lowest 5-year survival rate, at 5.4% in the UK, with a life expectancy of 2-6 months after diagnosis.^[84] Pancreatic adenocarcinomas (PaCa) are cancers of the pancreatic ducts, accounting for 95% of all pancreatic cancers.^[84] PaCa is characterized by aggressive tumour growth in addition to increased fibroblast and collagen-rich ECM.^[57] LOXL2 is up-regulated in human pancreatic cancer and shown to have a 7-fold increase of LOXL2 expression in PaCa than healthy human tissue.^{[30][70]}

Examination of tissue from 80 patients with pancreatic cancer determined that 82.1% were LOXL2-positive,^[70] and these patients had a significantly higher rate of metastasis. Additionally, protein expression in four pancreatic cancer cell lines; MIA-PaCa2, PANC1, AsPC-1 and BxPC-3 were explored.^[70] LOXL2 protein was expressed in MIA-PaCa2 and PANC1 cell lines, however absent in

AsPC-1 and BxPC-3, and *vice versa* with *CDH1* expression, demonstrating the inverse relationship. All four cell lines however showed SNAIL expression in varying degrees. This suggests that SNAIL expression is not only dictated by LOXL2 but by alternative signalling pathways. Using cellular migration studies using transepithelial techniques^[70] and despite having no LOXL2 expression, BxPC-3 had a significantly higher number of migratory cells than other cell lines. Furthermore, AsPC-1 showed a similar migratory potential to the cell lines which were LOXL2 positive. This result was unexpected and suggested that further signalling regulators are involved in the migration status of cells. Additional western blots showed activated FAK and Src to be upregulated in these cells. This indicates that cellular migration is also regulated *via* a LOXL2-independent pathway and therefore targeting LOXL2 may be ineffective in the treatment of metastasis.

3.4. Colorectal Cancer

Colorectal cancer, also known as bowel cancer, is the 4th most common cancer in the UK.^[85] Although 54% of colorectal cancers are potentially avoidable through changes in lifestyle factors, colorectal cancer may also arise due to the activation of proto-oncogenes such as KRAS and BRAF.^[86] Immunohistochemistry of 223 patient tissue samples showed only 28 of the samples had high LOXL2 expression. Despite the small proportion of samples with high LOXL2 expression, these patients had a significantly increased rate of distant metastasis and decreased rate of survival ($p = 0.024$).^[72] Assessment of LOXL2 protein and mRNA expression in 5 different colorectal cancer cell lines found that both protein and mRNA levels of LOXL2 were upregulated in only the SW480 cell line. Wound healing assays were conducted to assess the migratory potential of the cell lines showed that SW480 cells were the only cells to completely colonize the gap, denoting them the highest migratory potential, in comparison to other cell lines.

To ascertain whether LOXL2 is responsible for the difference in cellular migration behaviour, LOXL2 expression was knocked down in SW480 cells and upregulated *via* plasmid transfection in the other cell lines. The SW480 cell line showed a decreased migratory potential, whereas, the other cell lines exhibited an increase in motility, further supporting the initial findings.^[72] This indicates that there is an association between LOXL2 expression and migratory potential in colorectal cancer cell lines, unlike PaCa cell lines.^{[70][72]}

3.5. Oesophageal Squamous Cell Carcinoma

Oesophageal squamous cell carcinoma is the most common histopathological form of oesophageal cancer. Upon diagnosis, it is often found that the cancer has already metastasized, resulting in poor survival rates; in the UK, only 15.1% of adults with oesophageal cancer survive >5 years.^{[78][87]} Investigations of the significance of the LOX enzymatic family in oesophageal squamous cell carcinoma determined that the role of LOXL2 in esophageal SCC (ESCC) was unclear however intracellular LOXL2 was a predictive biomarker for poor prognosis; patients with decreased expression of nuclear LOXL2 but increased cytoplasmic LOXL2 expression had significantly lower survival rates.^[78]

SYMD3 is an enzyme which dimethylates and trimethylates H3K4, thereby activating downstream transcriptional events. Patients who had a high expression of SYMD3 had a significantly lower overall survival rate than those who exhibited low expression of SYMD3 ($n = 131$, $p = 0.008$).^[88] Furthermore, the knockdown of SYMD3 expression in nude mice that were transfected with the oesophageal squamous cell carcinoma cell line, KYSE150, inhibited local invasion. The interference of SYMD3 expression decreased the transcription and translation of LOXL2, showing a positive correlation. These results suggest that LOXL2 is a downstream signalling molecule of SYMD3 and could, therefore, be the effector protein responsible for the invasive profile of tissues with high SYMD3 expression. Confirmation of this would implicate LOXL2 as key component and regulator of metastasis.

LOXL2 is co-localised with actin filaments within oesophageal squamous cell carcinoma cell lines, KYSE150 and SHEEC, thereby impeding the architecture of the cells.^[79] These findings further

support the notion that LOXL2 plays a key role in invasion through the disruption of cytoskeletal components.

Although the data for the role of LOXL2 in invasion for oesophageal squamous cell carcinoma is very promising thus far, it is further complicated by the discovery of splice variants of LOXL2.^{[89][90]} A truncated form of LOXL2 was identified *via* cDNA cloning and was found to be localized and retained to the cytoplasm, unlike wt-LOXL2.^[90] This change in structure of LOXL2 results in a conformational change, thereby inhibiting its secretion and enzyme activity. Furthermore, this form of LOXL2 promoted cellular migration *via* activating different signalling pathways through chemokine upregulation.^[90]

The discovery of these proteins further complicates targeting LOXL2 due to changes in structure, site of action and mode of action, which may render anti-LOXL2 agents ineffective. However, LOXL2 mutants, which are enzymatically inert, activate EMT *via* SNAIL and FAK/Src pathways.^[40] This therefore suggests that the LTQ region is not involved in tumour metastases as previously expected.

3.6. Head and Neck Squamous Cell Carcinomas (HNSCC)

Exploration of the gene expression profile of SCC cell line (HaCa4) after LOXL2 or SNAIL knock down revealed that LOXL2-silenced cells had an upregulation of epidermal differentiation genes. Both LOXL2 and SNAIL knockdown cells reduced *in vivo* invasion, correlated with malignant progression in a mouse carcinogenesis model. Upregulated expression of both LOXL2 and SNAIL led to local recurrence in 256 human laryngeal squamous cell carcinomas. High levels of LOXL2 is a poor prognosis indicator associated with decreased overall and disease-free survival in laryngeal squamous cell carcinomas, lung squamous cell carcinoma, and lymph node-negative (N0) breast adenocarcinomas.^[91]

Using both gain and loss of function mouse models, it was found that *Loxl2* germ-line deletion led to 50% lethality in newborn mice (through heart defects) and *Loxl2* overexpression led to male sterility (through fibrosis, inflammation and epithelial disorganisation). Under chemical skin carcinogenesis conditions, *Loxl2*-overexpressing mice increased tumour burden and malignant progression, whilst *Loxl2*-deficient mice produced the opposite effect. LOXL2 levels in premalignant tumours showed a negative correlation with Notch1, furthermore LOXL2 is a direct repressor of *NOTCH1*. A link between LOXL2 expression and the NOTCH1 pathway in HNSCC was demonstrated.^[92] LOXL2 has also been shown to be a marker of poor survival in oral SCC, through attenuation of cancer growth in conjunction with the small molecule LOXL2 inhibitor, PSX-S1C in a mouse model.^[93]

3.7. Gastric Cancer

LOXL2 has also been studied in gastric cancer,^[94] with a correlation between survival and stromal cells which stained positive for LOXL2 in 548 gastric cancer patients. There was a significant reduction in the Kaplan-Meier survival rate for patients who tested positive for LOXL2 in both stromal and cancer cells ($p < 0.001$).^[94]

LOXL2 was shown to promote gastric carcinoma *in vivo*; gastric cancer cell line, BGC823 were injected into nude mice, then treated with an anti-LOXL2 antibody. High doses of antibody markedly reduced lung metastasis in the mouse model. Similar results were obtained in the spontaneous metastatic *in vivo* model using PAMC82-P3 cell line; the antibody inhibited growth and spontaneous metastasis.^[44] These findings are supportive of evidence that indicates extracellular LOXL2 to be involved in metastatic progression.

3.8. Lung Cancer

Lung cancer is the largest contributor to cancer deaths in the UK, predominantly due to metastasis, accounting for a fifth of all cancer-related deaths.^{[44][95]} Non-small cell lung cancer (NSCLC) accounts for 87% of all lung cancers and can be further distinguished by cell type; adenocarcinoma, squamous cell carcinoma and large cell carcinoma.

LOXL2 mRNA expression in human NSCLC samples were examined and compared to normal lung tissue.^[96] It was found that LOXL2 was significantly overexpressed in NSCLC samples compared to normal tissues; cytoplasmic and nuclear LOXL2 were negligible in healthy lung tissue whereas, in NSCLC specimens, LOXL2 levels were elevated. Furthermore, this increased level of LOXL2 was associated with an increase in tumour size and stage of cancer in adenocarcinoma and squamous cell carcinoma subgroups.^[96] This is further supported by silencing of LOXL2, inhibited migration and invasion in lung squamous cell carcinoma cell lines.^[97]

Investigations into the underlying mechanism involved in tumour progression in NSCLC have uncovered endogenous miRNA's (mi-Rs) to be directly involved in LOXL2 expression. Mi-Rs are small non-coding RNA which bind to the 3'-untranslated region of genes, consequently cleaving or repressing the translation of its target gene.^[98] Loss of mi-R function has been reported to result in tumour progression and therefore have a tumour-suppressor effect.^{[99][100]}

Quantitative RT-PCR and western blot analysis verified that restoring miR-29 expression, LOXL2 expression was significantly downregulated in squamous carcinoma cell lines.^[97] This identified another molecular pathway in which LOXL2 is implicated.

Other mi-Rs also regulate LOXL2 expression; mi-R504 has been identified^[98] to have inhibitory effects on migration in lung adenocarcinoma cell lines (A459), however, these effects were reversed when LOXL2 was overexpressed. LOXL2 expression has been demonstrated^[101] to be directly regulated by the miR-200/ZEB1-axis induced EMT. However, when LOXL2 is overexpressed, no effect on metastatic potential was observed, indicating that LOXL2 alone is insufficient to drive metastasis, but is essential for growth in the tumour microenvironment.^[101] This suggests that LOXL2 mediates the effects of other signalling molecules rather than being the effector protein, therefore LOXL2 would be a poor target for treatment of this metastatic disease.

3.9. Clear Cell Renal Carcinoma

Clear cell renal carcinoma (ccRCC) is the most common form of renal cancer, accounting for 80% of all cases.^{[99][102]} At diagnosis, around 30% of renal carcinomas have already metastasized and neither radiotherapy nor chemotherapy is used for treatment. Moreover, LOXL2 is significantly upregulated in renal carcinoma compared to normal tissue ($p = 0.0001$).^[100]

As with NSCLC data, the mi-R29 family have been identified in ccRCC, maintaining the same role; restoring levels inhibits migration.^{[99][102]} Quantitative RT-PCR and western blotting conducted on kidney cancer cells, 786-O and A498 lines assessed the effects on LOXL2 expression. In both cell lines, LOXL2 expression was significantly reduced with treatment of mi-R29.^[99] Similar results were obtained^{[102][100]} relating the LOXL2 expression to the restoration of mi-R26s function. These findings highlight a compelling pathway linking LOXL2 with mi-RNAs in cancer metastasis and its underlying biology; essential for understanding the mechanism of LOXL2 action in cancer.

4. Modulation of LOXL2 activity

4.1. Small molecule approaches to LOXL2 inhibition

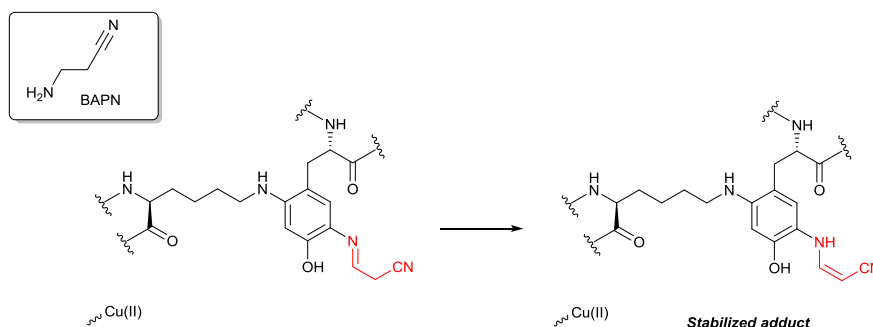
It was argued by Rowbottom and colleagues that small molecule inhibitors may be more successful in inhibiting LOXL2 than antibodies. Small molecules can target both intracellular LOXL2 and extracellular LOXL2.^[41] Additionally, they are sufficiently small to enter the active site of the enzyme which subsequently leads to its inhibition. As antibody approaches have failed in fibrotic diseases, another argument presented for the use of small molecule inhibitors, is their ability to pass into fibrotic tissue in the ECM compared to antibodies.^[41] Even though small molecule inhibitors may be better suited to target diseased tissues, this is yet to be conclusively shown in human studies to determine if there is a positive impact on disease free progression.

In the following section, a timeline of LOXL2 inhibitors is discussed beginning with BAPN, through to selective, designed LOXL2 inhibitors which have entered clinical trials.

β -aminopropionitrile

β -Aminopropionitrile (BAPN) is a small molecule pan-inhibitor of the LOX family of enzymes. It competitively and irreversibly inhibits the catalytic domain of LOX enzyme which is often used in *in vitro* and *in vivo* studies to inhibit the activity of LOXL2. As BAPN is non-selective for LOXL2, it is currently approved by the FDA for use in humans for investigational purposes only.^[103] It has not been deemed suitable for clinical use thus far, however, LOXL2 specific small molecule inhibitors are in the process of being identified.^{[20][41]}

The simple structure of BAPN contains a primary amine and a nitrile group within its structure (**Scheme 2**), the amine mimicking the role of lysine in reacting with the LTQ cofactor.



Scheme 2. BAPN (in red) bound covalently and irreversibly to LTQ cofactor in LOXL.^[20]

Unlike lysine, BAPN forms a stable product with LOXL2 through tautomerisation (**Scheme 2**). The nitrile group of BAPN hydrogen bonds to the enzyme, further preventing the LTQ cofactor of LOXL2 from regenerating.^[20]

The study of BAPN in various diseases and cellular processes related to LOX family inhibition is shown in **Table 2** both *in vitro* and *in vivo*. BAPN was injected intraperitoneally in a mouse model to stabilise oncogenic stressed-induced senescence and mice survival increased with BAPN due to LOX inhibition (**Table 2**, Entry 1).^[104] BAPN has been tested for prostate cancer where treatment was performed before and after the implantation of tumour (**Table 2**, Entry 2).^[105] There was a reduction in tumour growth when treatment was commenced prior to tumour implantation in mice. On the other hand, for treatment post-implantation there was an increase of tumour growth and no impact on spontaneous formation of lymph node metastasis.^[105] This *in vivo* experiment suggested that BAPN has a role in tumour stroma or surrounding epithelium as LOX does not have a direct impact on tumour cell viability.^[105] BAPN has also been tested in IPF, reducing stiffness and returning collagen remodelling to baseline levels (**Table 2**, Entry 3).^[106] Furthermore, LOX, LOXL2 and LOXL3 were identified at higher levels in human donor vessels of those diagnosed with idiopathic pulmonary arterial hypertension.^[107] The expression levels of these enzymes were similar in mice, and a higher level of LOX resulted in vascular lesions. BAPN treatment in mice normalised the matrix architecture and brought the right ventricle systolic pressure down to baseline levels.^[107] Likewise, when BAPN was used in renal cell carcinoma of the cell line A498 (**Table 2**, Entry 4), the migration of these cells were halted, showing that LOX and LOXL2 influenced cell migration.^[107]

More recently, BAPN has been tested locally in humans for hypertension *via* intradermal microdialysis (10 mM dose) (**Table 2**, Entry 5).^[103] There is a higher level of LOX when vascular stiffness increases, resulting in microvascular dysfunction.^[103] Expression levels of both LOX and LOXL2 differed according to age and hypertension state.^[103] Soluble LOXL2 was higher in the 20-27 age group than both normotensive and hypertensive groups which were both similar. For soluble LOX, there were no differences between these groups. Soluble LOXL2 was less expressed in the hypertensive group than the 41-63 years old normotensive group. However, the matrix-bound LOXL2 was higher in hypertensive than normotensive group.^[103] The different expression patterns implied

that the LOX family members have different roles in microvascular function relating to changes in blood pressure and age.^[104]

There is controversy regarding the selectivity of BAPN. Several research groups have stated that BAPN does not target LOXL2 selectively.^{[23][108][109]} It was identified in different breast cancer cell lines that BAPN blocked LOX activity in MDA-MB-231 cells however, not in MDA-MB-435 cells as they expressed LOXL2 (**Table 2**, Entry 6).^{[108][110][111]} Conversely, several papers state that BAPN targets LOX and all the other LOX isoenzymes^{[103][111]} with equipotency.^{[49][112]} Additionally, there are other articles which indicate that BAPN preferably inhibits LOXL2 over LOX in a dose dependent manner.^[69] Hajdú and co-workers recently stated that the physiological roles of LOX are not accurate and misleading conclusions have been made in previous literature reports.^[23] It has been recommended that further screening of known inhibitors developed so far on all of the enzymes in the LOX family, in order to avoid confusion of the results reported in the literature to date.^[23]

Additionally, it is not clear whether BAPN has a transcriptional role and affects the *mRNA* levels of LOX. It was indicated that BAPN did not affect the steady state levels of *mRNA* in lung fibroblasts and therefore BAPN is only responsible for inhibiting the enzymatic activity of LOX (**Table 2**, Entry 7).^[113] In another study, mice treated with BAPN for pulmonary hypertension had a decreased expression of LOX *mRNA* and therefore BAPN has a dual role of decreasing the expression levels of LOX *mRNA* and inhibiting enzymatic activity (**Table 2**, Entry 8).^[114]

Non-selective inhibitors, such as BAPN, can result in serious adverse effects as the expression patterns of the enzymes in diseased and healthy tissue differs, depending on the member of LOX studied. LOX is specifically reported to have a similar expression level between diseased and healthy tissue whilst LOXL2 is greater expressed in diseased tissue.^{[23][74]} Lack of LOX resulted in severe side effects in mice such as pulmonary and cardiovascular failure.^[41] LOXL2 has a higher expression level in diseased tissue rather than in healthy tissue,^[23] therefore selectively inhibiting LOXL2 should result in a less severe side effect profile than inhibiting LOX.^{[23][74]} Further, it has been documented that eliminating LOX expression resulted in a higher risk of aneurysms as the blood vessels loses their integrity.^[103] Hence, no human study has been conducted where BAPN has been given systemically to investigate the complete blockade of the LOX family as it can lead to a spontaneous coronary artery dissection.^[103]

BAPN is a sub-optimal LOXL2 inhibitor and is unsafe to be used systemically. However, there has been success with BAPN as a tool compound to inhibit pan-LOX enzymes and positive outcomes have been seen in cancer and fibrosis model studies. BAPN has been used as a starting point to identify new chemical scaffolds by altering the design to produce a more selective inhibitor against LOXL2.^[20]

Table 2. A summary of indications that BAPN has been studied.

| Entry | Disease Type/LOX involvement in cellular processes | Experimental Model | Dose of BAPN | Ref. |
|-------|--|--|--|-------|
| 1 | Oncogenic stress response and tumorigenesis | Human epithelial cell | 500 μ M for LOX activity assay | [104] |
| 2 | Prostate cancer | Wild-type and Pdx1-Cre, LSL-Kras ^{G12D/+} , INK4a/Arf ^{lox/lox} mice Dunning R-3327 AT-1 rat prostate tumour cells | 100mg/kg administrated intraperitoneal injections 100 mg/kg administrated by intraperitoneal injections | [105] |
| 3 | Idiopathic pulmonary fibrosis | Copenhagen rats Human lung parenchymal tissues and primary human lung fibroblast | 100 μ M | [106] |
| 4 | Tumour cell migration | A498 cells | 300 μ M | [107] |
| 5 | Microvascular function | Human participants | 10 mM administrated by intradermal microdialysis | [103] |
| 6 | Breast cancer | Forearm skin tissue Human MDA-MB-231 and MDA-MB-435 cells | 200 μ M (cellular) | [108] |
| 7 | LOX expression influence transcriptome of lung fibroblasts | SCID mice Primary lung fibroblast from C57BL/6J mice New born mice models exposed to 85% oxygen for the first 19 days | 1.0 mM (fibroblasts) 15 mg/kg/day (mice) | [113] |
| 8 | LOX in vascular remodelling in pulmonary arterial hypertension | Mice | 150 mg/kg/day administered intraperitoneally | [114] |
| 9 | Oral fibrotic conditions | Gingival tissue from human subjects | 500 μ M | [49] |
| 10 | Renal fibrosis | Col4a3/Alport mice | 300 μ M was used as a control in the assay of plasma LOXL2 inhibition | [115] |
| 11 | Keratinocyte differentiation | HaCaT cells | 0.2 mM | [116] |
| 12 | Collagen and elastin | Bovine neck ligament elastin, calf skin collagen type I and type III and human placenta collagen type IV were substrates for the recombinant LOXL2 assay | 1.0 mM | [109] |
| 13 | Modulating LOXL2 activity | Recombinant LOXL2 | 0.001 mM, 0.01 mM and 0.1 mM | [69] |
| 14 | Uveal melanoma | 92.1, SP6.5, MKT-BR and OCM-1 cell lines | 750 μ M/l | [117] |
| 15 | Wilson's Disease | COS-7 Green monkey kidney cells | 0.05 mg/ml | [111] |
| 16 | Hepatocellular carcinoma | SK-HEP-1 and HLF cell lines | 350 μ M | [118] |
| 17 | Breast Cancer | Recombinant LOXL2 protein and native human LOX enzymes. PIC50 test compared the selectivity of the newer PXS compounds. | Not stated | [119] |
| 18 | Breast Cancer | MCF-7 cells, MDA-MB-231 cells and MDA-MB-231 xenograft model | 100 μ M (cells) 100mg/kg (mice) | [120] |
| 19 | LOXL2 role in pathological microenvironment | MDA-MB-435 xenograft model | 100 mg/kg | [30] |
| 20 | LOX involvement in human placental | Extravillous cytotrophoblasts | 100 μ M or 200 μ M for the | [121] |

| | | | | |
|----|--|---|---|-------|
| 21 | cytotrophoblast invasion LOX distribution in the activity of keratoconus corneas | Corneal fibroblast | invasion assay 500 mM for LOX activity assay | [122] |
| 22 | Disturbance of LOX1 and elastin metabolism in glaucoma development | Primary human optic nerve head astrocytes | 50 µg/ml | [123] |
| 23 | Determining if the expression of LOX genes is regulated by TGFβ | Trabecular meshwork cells | 1 mM, 3 mM, 10 mM and 30 mM | [124] |
| 24 | Purification of enzymatically active hLOX and LOXL from E. coli to make large quantity of enzyme | Amine oxidase assay using LOX and LOXL protein | 10 M and 100 M | [110] |
| 25 | Breast cancer | MCF-7, T47D, Hs578T, and MDA-MB-231 cell lines | 100 µM and 200 µM | [51] |

D-penicillamine

Wilson's disease is a genetic disorder related to the metabolism of copper which results in the accumulation of copper, as there is a decreased biliary excretion of this metal.^[114] A study predicted there are in the region of 2,000 people with Wilson's disease in the UK.^[114] Symptoms of Wilson's disease include neurological complications which can lead to stiffness and tremor.^[112] The disease also affects the liver with clinical signs such as cirrhosis and hepatitis.^[112] Biliary obstruction has also been documented in Wilson's disease.^[112] D-penicillamine (D-pen) (**Figure 5**), a copper chelator is used in the treatment of Wilson's disease.^{[111][114][112][125]} Additionally, D-pen does not have antifibrotic properties but has been shown to decrease the fibrosis rate of certain organs such as the skin.^[126]

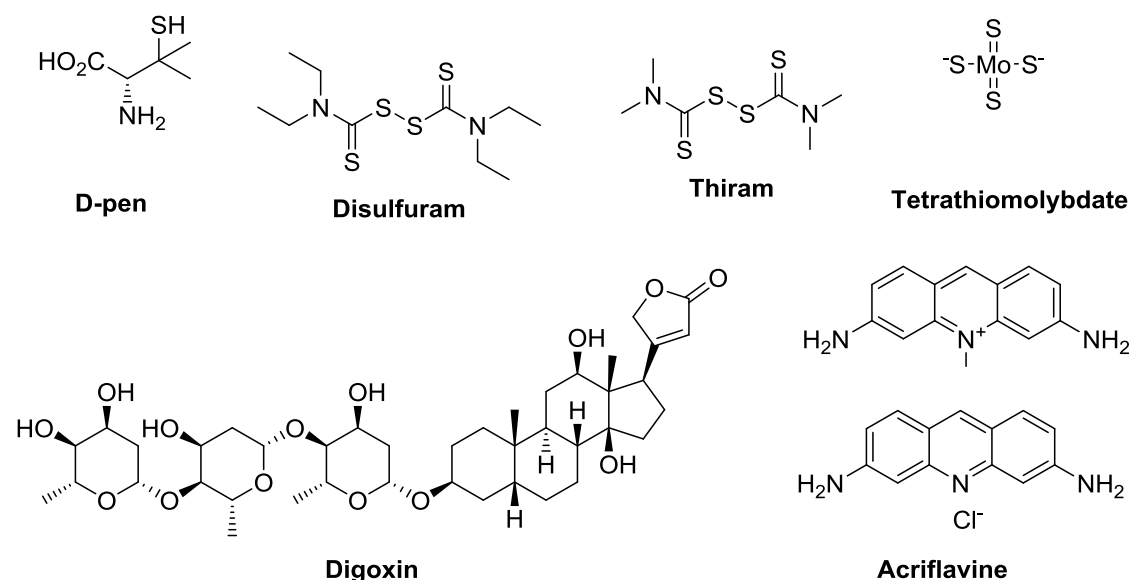


Figure 5. Examples of early LOXL2 inhibitors.

D-pen is an orally administered drug which is rapidly absorbed through the intestines and has a peak concentration at around 1-3 h in the plasma.^[127] It is > 80% bound to plasma proteins and is eliminated in a biphasic manner with an initial elimination of $t_{1/2} = 1-3$ h which is followed by a slower phase. This implies that it is stored in the tissue and slowly released.^[127] Differing to BAPN, D-pen has a secondary amine (**Figure 5**). The mechanism of action is not clearly understood which has led to inconsistencies in the literature as to whether D-pen interacts with the LOX enzymes.^[126]

D-pen has been shown to stop the oxidation of LOXL2 in human liver cancer HEPG2 cells.^[111] D-pen has not been extensively studied for the inhibition of LOXL2 however, D-pen has been shown to block the active site of both LOX and LOXL2.^[128] Another study of D-pen investigated the activity of LOXL2 and described D-pen to be an inhibitor of LOXL2.^[55] It was concluded that D-pen did not have an effect on the rate of growth of the tumour in 4T1 cells.^[55] The mice model used, MMTV-PyMT, had less metastases in the lung when treated with D-pen and was more useful when used earlier in the disease progression.^[55]

However, this result was contradicted by the finding that D-pen does not inhibit LOX directly but rather has a role in blocking the crosslinking of collagen.^[129] It was thought that the mechanism of D-pen is linked to the lack of crosslinks being formed between collagen and elastin than its interaction with the LOX family,^[126] forming thiazolidine rings by binding to the allysine residue in the collagen fibres. The thiazolidine ring formed sterically blocks the condensation of allysine residues in neighbouring collagen strands.^[126]

D-pen has also been tested in disease states with higher levels of LOXL2, for instance cholangiopathies.^[130] Cultured murine biliary epithelial cells were treated with 150 μ M D-pen to inhibit the activity of LOX enzymes, alongside 150 μ M bathocuproinedisulfonic acid.^[130] The main outcome does support the use of anti-LOXL2 therapy in primary sclerosing cholangitis (PSC), however potential consequences such as bile duct injury should be considered.^[130] It is important to highlight that D-pen was clinically tested in patients with primary sclerosing cholangitis in the 1980s and the conclusion was that D-pen was not an effective treatment in this disease.^[131]

Recently, D-pen was described to be a pan-LOX family inhibitor rather than a specific inhibitor of LOXL2.^[23] Despite some conflicting data regarding its mechanism of action, it can be concluded that D-pen does not selectively inhibit LOXL2.^[23] Therefore, this drug is sub-optimal for the inhibition of LOXL2.

Escin Ia from SFAC of *Aesculus chinensis*.

Aesculus chinensis Bunge fruits, has sparked interest in cancer therapy, due to its interaction with LOXL2. It is a deciduous tree with origins in China, with claims that a fraction of the fruits, SFAC, has anti-cancer properties. When tested in MDA-MB-231 cells, SFAC showed a decrease in LOXL2 mRNA expression and an increase in E-cadherin expression. Furthermore, it was identified that Escin Ia constituent (**Figure 6**) of SFAC was responsible for this activity, and when isolated, Escin Ia decreased the number of motile MDA-MB-231 cells.^[120] Escin Ia has also been tested in xenograft mice models that were injected with MDA-MB-231 cells into the fat pads of the mammary glands. The mice were either untreated or treated with 2 mg/kg Escin Ia, 4 mg/kg Escin Ia or BAPN 100 mg/kg as the standardised control. After five weeks treatment, mRNA expression was assessed and quantified. The results of three independent experiments showed that Escin Ia had increased potency compared to BAPN and had reduced expression of LOXL2 and SNAIL, in addition to increasing the expression of E-cadherin but were not statistically significant. However, Escin Ia did significantly reduce the mRNA expression of Slug and Zeb1, EMT transcription factors. Although these findings do not show significant inhibition of LOXL2, Escin Ia may be effective as an adjuvant therapy to LOXL2 inhibition.

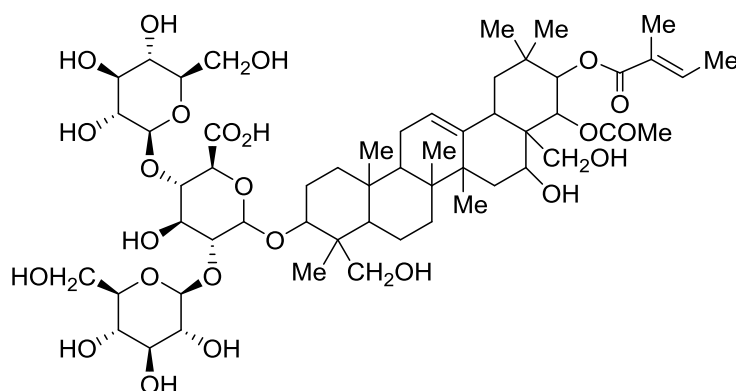


Figure 6. Structure of Escin Ia (absolute stereochemistry undefined) from SFAC of *Aesculus chinensis*.

Other LOXL2 inhibitors

Tetrathiomolybdate, a copper chelator, has been reported to stop the expression of activated LOX, LOXL1 and LOXL2 in pulmonary fibrosis of mice.^[132] Furthermore, digoxin and acriflavine were compounds identified which stopped the expression of LOX proteins and hypoxia induced mRNA of LOX and LOXL4 in MDA-231 cells and LOXL2 in MDA-435 cells.^[108] These compounds will not be further discussed in this review, as these compounds have only been reported to control the expression levels of LOXL2 rather than directly modulating enzymatic activity.

4.2. Next Generation Small Molecule Inhibitors of LOXL2

In recent years, numerous patents have been released for small molecule inhibitors against LOXL2, the year-on-year trend is highlighted in **Figure 7**.

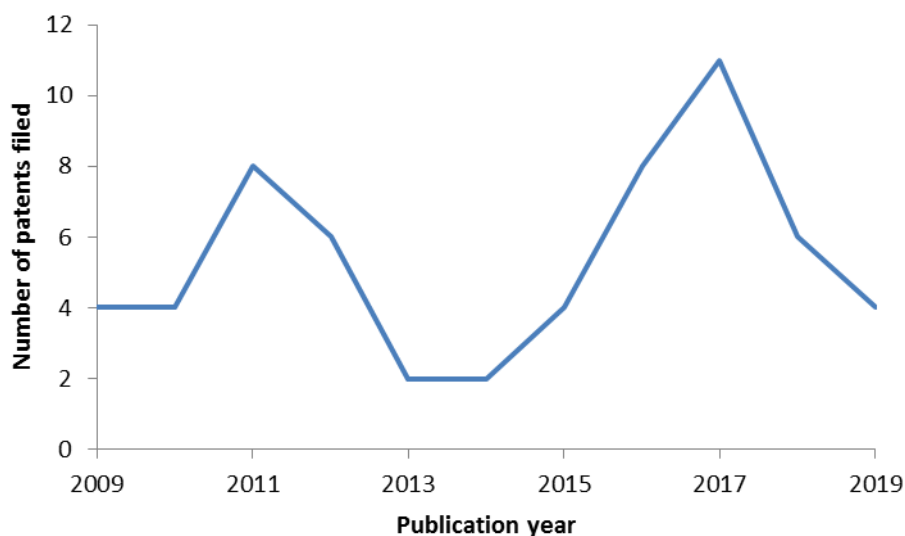


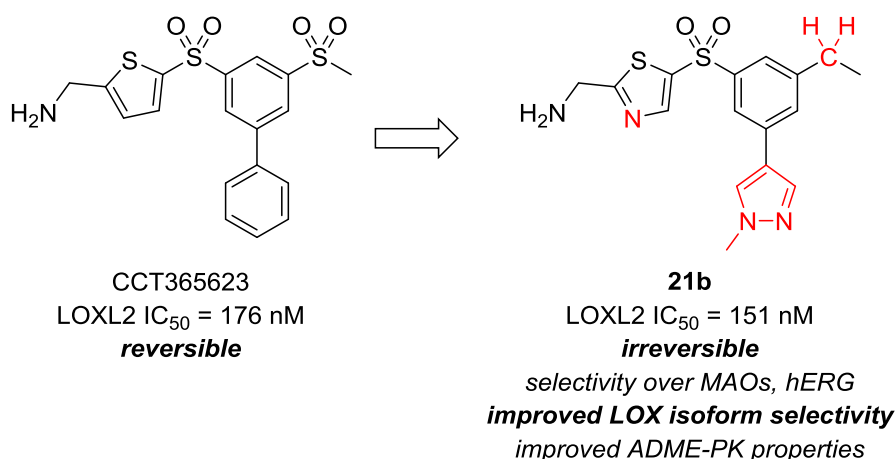
Figure 7. The number of patent filed/per year describing the small molecule inhibition of LOXL2 (data until August 2019)

A renaissance in the the development of commercially competitive small molecule LOXL2 inhibitors over the past decade is shown in **Figure 7**. These developments, coupled with the recently characterised *h*LOXL2 precursor crystal structure,^[22] suggest the field can only be expected to rapidly grow. There is also compelling evidence that LOXL2 is related to cancer and fibrosis which makes this an attractive target without a competitor compound on the market as of yet. Patent protected small molecule inhibitors of LOXL2 have been developed (**Table 3**).

There are limitations to the scope of biological interrogation reported for these small molecules in the patent literature. Exemplar compounds were selected from the disclosed patents based on the hypothesis of potency or significant *in vivo* testing reported.

Compounds from Entries 1 and 4 (**Table 3**) were chosen as the most potent examples and no further biological testing was reported for the other compounds presented in their respective patents.^{[133][134]}

For Entry 2 (**Table 3**), there was further biological testing conducted on the example compound selected within this patent literature.^[135] The main results being a dose dependent decrease of mature crosslinks.^[135]



Scheme 3. Development of CCT365623 into a potent, irreversible, LOXL2 selective inhibitor (**21b**).

In vivo pharmacokinetics tests were conducted on the example compound from Entry 3 (**Table 3**), recently revealed as CCT365623,^[136] using a mouse model dosed at 10 mg/kg intravenously and 50 mg/kg orally.^[137] A rat was also dosed at 4 mg/kg intravenously and 20 mg/kg orally.^[137] The results showed for the mouse that the area under the curve (AUC) was 15 μ M/h, maximum concentration (C_{max}) was 17 μ M and the bioavailability was 45% with a half-life of 0.6 h. For the rat model the AUC was 0.22 μ M/h, C_{max} was 0.34 μ M however, the bioavailability was not documented. A half-life of 0.5 h for the rat was reported.^[137] Tumour model studies conducted in PDAC R172H (mouse pancreatic carcinoma), MDA-MB-231 and MMT-PyMT breast transgenic model where the primary tumour (ratio of tumour volume (treated)/ tumour volume (control)) were 0.53, 0.46 and 0.40, respectively.^[137] The exemplar compound (**Table 3**, entry 3) was also tested in MMT-PyMT breast transgenic metastatic model with a metastasis of 0.57 (ratio of metastatic surface area (treated)/ metastatic surface area (control)).^[137] Within the metastatic model the lung metastases were counted.^[137] There were 6 mice in each treatment and control (treated with DMSO) group. In the control group there were a total of 18 small, 15 medium and 34 large metastases.^[137] In the group of where the mice were treated with the example compound there were 1 small, 4 medium and no large metastases. The small metastases were <100 μ m, medium were 100 μ m-200 μ m and the large metastases were >200 μ m.^[137] The results therefore highlight that this orally delivered compound has promising properties for the treatment of metastatic breast cancer.

The first report of CCT365623 (without a disclosed structure) detailed its properties as an inhibitor of LOX.^[138] CCT365623 was shown to disrupt epidermal growth factor receptor (EGFR) cell surface retention and slowed the growth of both primary and metastatic tumours *in vivo*. This work validated the potential of LOX inhibition as a therapeutic avenue with a small molecule approach.

Recently the medicinal chemistry campaign that delivered CCT365623 from a high-throughput screen has been disclosed.^[136] In particular, the key aminomethylenethiophene (AMT) moiety proved a potent LOX inhibitor scaffold but with a poor pharmacokinetic profile. Refinements to the structure retained the LOX potency but addressed the lack of oral bioavailability, resulting in CCT365623. Selectivity for LOX over LOXL2 remained poor at 1.6 fold.

Most recently, CCT365623 has been further refined to structure **21b** (**Scheme 3**) with enhanced LOXL2 selectivity (now 22-fold) and pan-MAO, SSAO and hERG selectivity of at least 189-fold (SSAO). Key to these improvements was the replacement of the thiophene core with a specific thiazole regioisomer. This also changed the profile of the compound from a reversible to an irreversible inhibitor. In summary **21b**, coupled with the improved PK profile and LOXL2 selectivity, demonstrated anti-tumour efficacy in a LOX-dependent GEMM breast cancer model.^[139]

The exemplar compound in Entry 5 (**Table 3**), was tested in a mouse model. It showed a reduction in cancer tongue volume and metastasis of cancer in oral metastatic cancer within a mice model. Promisingly, this compound has shown to reduce liver fibrosis when tested in a mouse model.^[140]

A collection of diazabicyclo[3.2.2]nonanes, intriguingly with a *des*-primary amine group, have been patented as LOXL2 selective inhibitors (**Table 3**, entry 6).^[141] Selected compounds were tested against a transgenic mouse breast cancer model at 200 mg/kg po qd, with statistically significant ($p < 0.05$) reduction in breast cancer metastasis to the lungs.

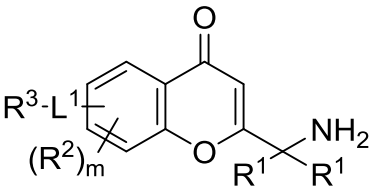
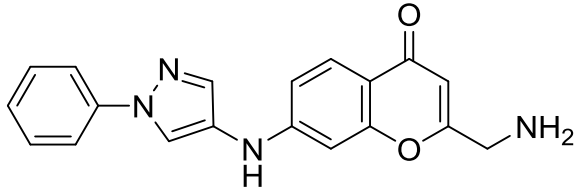
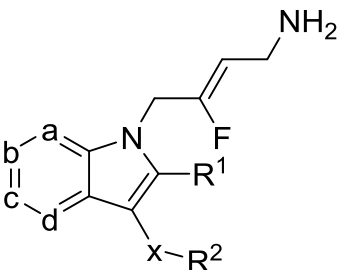
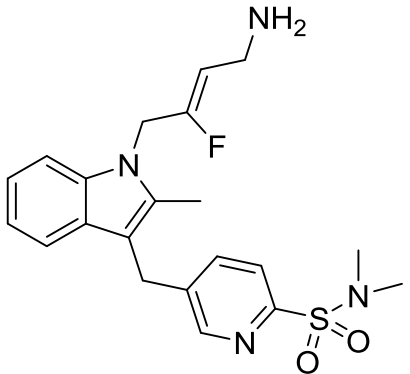
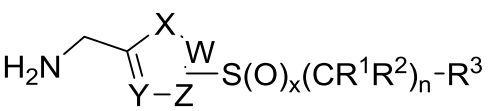
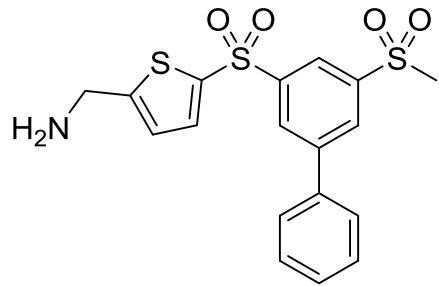
Pharmaxis has developed a selective inhibitor of LOXL2 that is in pre-clinical testing (**Table 3**, entry 7).^[119] However, the structure of this specific drug has not been disclosed. The compound is referred to as PXA-S2B and the oral pro-drug is known as PXS-S2A.^[119] PXS-S2A is reported to have good oral bioavailability, good plasma stability, moderate plasma protein binding and high metabolic stability.^[119] PXS-S2A was identified through the modification of PXS-S1A which is described to be a first generation inhibitor with similar activity and selectivity profile to BAPN.^[119] The pIC_{50} is 6.8 and 6.4 for PXS-S1A and BAPN, respectively.

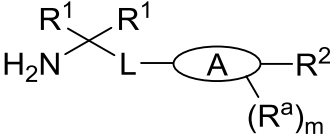
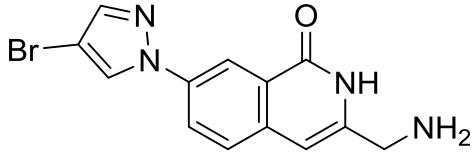
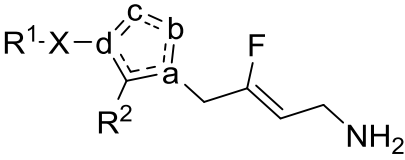
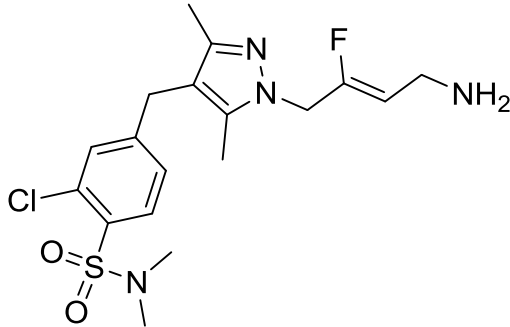
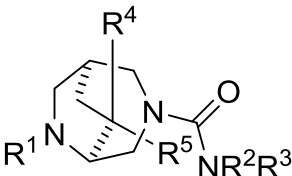
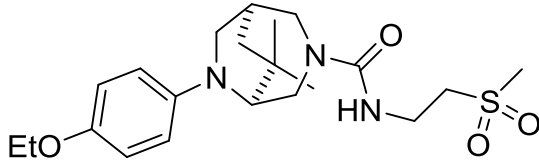
The pIC_{50} of PXS-S2A was 8.3 with greater selectivity for LOXL2 than PXS-S1A. In healthy mice PXS-S2B was dosed daily for 24 weeks and a tolerated dose of 10 mg/kg was identified.^[119] Additionally, PXS-S2A had a 500-fold selectivity for LOXL2 over the other human amine oxidases such as monoamine oxidases and diamine oxidases. PXS-S2A has been tested in a range of cell lines and was shown to inhibit proliferation, migration and invasion including the MDA-MB-231 cell line.^[119] This specific cell line is known to have a high level of LOXL2 expression.^[119] Both PXS-S1A and PXS-S2A inhibited cell growth in a dose dependent manner and the data demonstrated that both LOX and LOXL2 play an important role in the cell growth of breast cancer cells.^[119] These results were confirmed with *shLOXL2* treated cells, with decreased cell growth compared to the control (scrambled control cells). PXS-S2B was shown to reduce primary tumour growth with ~55% reduction, lower than PXS-S1A with a ~75% reduction of tumour growth.^[119] A press release stated these LOXL2 inhibitors completed phase I clinical trials and showed positive results at a range of doses and maintained a desired level of inhibition over 24 h, the underlying trial data is yet to be disclosed.^[142]

LOX has a role in breast cancer tumour growth and therefore using both inhibitors of LOX and LOXL2 showed a greater effect.^[119] Inhibiting LOX and LOXL2 had a significant reduction in metastasis in the liver and lung but this was not witnessed by inhibiting LOXL2 alone.^[119] However, when treated with PXS-S2A there was a reduction of vessel density which limited the blood supply to the primary tumour.^[119] Inhibiting early angiogenesis which is directed by LOXL2 resulted in the reduction of primary tumour growth. Using both inhibitors also reduced the expression level of α SMA significantly. This compelling data set, warrants further investigation of small molecule inhibitors of LOXL2 as a therapeutic approach in human cancers.^[119]

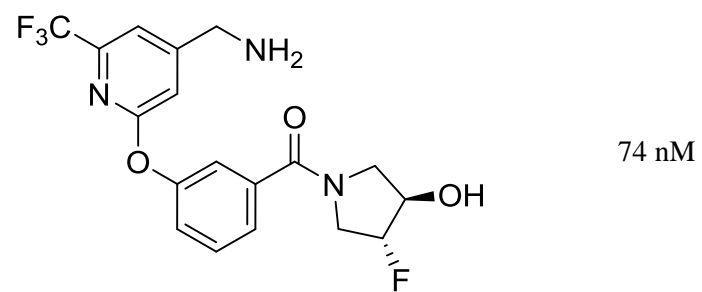
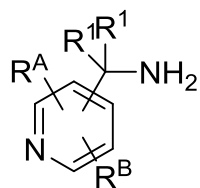
PXS-S2B has also been tested in mice for the treatment of renal fibrosis to determine if it has renal protective properties.^[48] A dose of 10 mg/kg was well tolerated with no signs of damage to the liver, heart and lungs of the mice.^[48] The staining proved that there was a higher expression of LOXL2 in diabetic mice, particularly in the glomeruli. The sign for diabetic nephropathy is the presence of albuminuria and this was reduced with PXS-S2B and further reduced with Telmisartan.^[48] Telmisartan is primarily used in the treatment of diabetic kidney disease as it blocks the renin-angiotensin-aldosterone system.^[48] The LOXL2 inhibitor was able to reduce the glomerulosclerosis score but Telmisartan did not do so significantly. Fibronectin *mRNA* was also reduced and this glycoprotein is an important ECM protein in this disease progression.^[48] Collagen I was also reduced which is usually higher in renal fibrosis.^[48] Inhibiting LOXL2 has resulted in renal protective properties by impeding processes which drive renal fibrosis. In summary, PXS-S2A is a promising drug for the targeted treatment of cancer^[121] and fibrosis.^[48]

Table3. The main results from patent and scientific literature, including an advanced exemplar compound.

| Entry | Patent company, [reference] | year, | Number of compounds | General Patent Formula Protected | Exemplar compound | IC ₅₀ (LOXL2) |
|-------|--|-------|---------------------|--|--|---|
| 1 | PharmAkea, Inc., USA. ^[133] | 2017 | 70 |  |  | <0.3 μM |
| 2 | Pharmaxis, Ltd., Australia. ^[135] | 2017 | 44 |  |  | <300 nM |
| 3 | The Institute of Cancer Research Royal Cancer Hospital, UK. ^[137] | 2017 | 164 |  |  | At 20 min 3.8 μM At 20 hours 0.26 μM |

| | | | | | |
|---|--|----|--|---|---------|
| 4 | 2018 Pharmakea Inc. Ltd, USA. ^[134] | 93 |  |  | <300 nM |
| 5 | 2018 Pharmaxis Ltd, Australia. ^[140] | 67 |  |  | <1 μM |
| 6 | 2019 The Institute of Cancer Research Royal Cancer Hospital, UK ^[141] | 85 |  |  | 1.0 μM |
| 7 | 2017 Pharmaxis, Ltd, Australia. ^[119] | - | - | PXS-S2A, PXS-S2B | |

8 2016 and 2018
PharmAkea, Inc.,
USA.^{[143][144][41]} 105



74 nM

The example compound discovered by PharmAkea in Entry 8 (**Table**) has a high affinity for LOXL2. It was shown that the aminomethyl pyridine moiety interacts with the active site of LOXL2. This interaction forms a pseudo-irreversible inhibitory complex which is time-dependent and progressed to the initial phases of clinical testing as a LOXL2 specific inhibitor.^{[143][144]} The discovery process of this compound is illustrated in (**Table 4**).

Table 4. A summary of the key results from the 4-(Methylamino)-6-(trifluoromethyl)-pyridine-derived class of LOXL2 inhibitors.^[41]

| Compound | Structure | <i>h</i> LOXL2 IC ₅₀ (μM) | blood | <i>h</i> LOXL2 cells IC ₅₀ (μM) | CHO clogP |
|-------------------|-----------|---|-------|---|--------------|
| I | | 0.87 | | 0.078 | 2.8 |
| II | | 0.71 | | 0.074 | 2.8 |
| III (PAT-1251) | | 1.15 | | 0.082 | 2.8 |
| IV | | 0.042 | | 0.062 | 4.3 |

It was found that using an electron withdrawing group such as -Cl on the pyridine led to an increase in potency compared to an electron donating group. Despite the slightly decreased potency following the addition of the -CF₃ group on the pyridine, it led to a higher selectivity towards LOXL2 and this is predicted to be due to the steric bulk.^[41] It was also found that analogues with relatively high clogP values are more prone to be bound to plasma proteins.^[41] Due to the clogP values, compound I has a plasma protein binding of 68% whilst compound IV has a plasma protein binding of 96%.^[41]

The C57BL/6 mice showed a better lung to plasma ratio of compounds with clogP equal to or greater than 2 (**Table 4**).^[41] Additionally to the clogP requirements, compounds with a pKa of 8 had better penetration into the lungs.^[41] The aminomethyl groups of the compounds illustrated in **Table 4** have a pKa ~8. Compound I was optimal as its clogP was within the lung penetrating range of 1.8 to 3 and it has a high potency in the human blood assay.^[41]

The racemic-trans version of the molecule (Compound I, **Table 4**) has undergone testing in a C57BL/6 mice model for lung fibrosis (oral dosing 3-60 mg/kg).^[41] A dose dependent reduction of lung fibrosis in mice was observed, the most promising results were seen with doses between 10-30 mg/kg.^[41]

The (*R,R*)-enantiomer (Compound III, **Table 4**) was chosen over the (*S,S*)-enantiomer (Compound II, **Table 4**) due to a slightly improved potency. Both enantiomers showed high selectivity towards LOXL2 when compared against LOX and the amine oxidase family.^[41] As LOXL2 does not appear to differentiate between either enantiomer, supports the theory that the pendent amide substituent

(compound IV, **Table 4**) is not involved in the key interaction with LOXL2 active site.^[41] The (*R,R*)-enantiomer (named PAT-1251) has progressed into phase I clinical trials.

PAT-1251 has limited potential for cardiotoxicity and showed negative results for genetic toxicity.^[41] There is also a low likelihood for liver toxicity and low risk for reactive metabolites to be formed.^[41] This breakthrough compound, PAT-1251 was the first small molecule inhibitor of LOXL2 to progress into clinical trials.^[84] The phase I trial was completed in November 2016 and the results have been reviewed.^[145] However, they are yet to be reported on ClinicalTrials.gov at the time of writing.^[145]

PAT-1251 (30 mg/kg) was also tested in Alport mice (Col4a3 null mice) for renal fibrosis to identify the role LOXL2 plays in this disease.^[115] The results from this treatment showed a decreased leukocytic and myofibroblasts accumulation in the mice.^[115] Using PAT-1251 further decreased fibrosis and glomerular sclerosis whilst the kidney function of mice also improved.^[115]

It was determined that LOXL2 is important in fibrosis disease progression in COL4A3/Alport mice.^[115] It was also identified that inhibiting LOXL2 is important in the stabilisation of the basement membrane in the kidney.^[115] The results are therefore promising in the treatment of fibrosis and reducing the disease progression using this small molecule inhibitor against LOXL2.^[115]

Furthermore, PAT-1251 (and related structures) are *pseudo*-irreversible inhibitors. This interaction is reported to prevent LOXL2 from becoming activated again as a strong Schiff base complex is formed.^[41] It was argued that using an irreversible inhibitor will allow for a longer duration of action despite the clearance of the drug from the body.^[41] This may overcome toxicity issues related to higher dose exposure over a prolonged period of time.^[41]

Small Molecule Inhibitors of LOXL2 in the academic pipeline

During routine testing of four pan-LOX inhibitors to identify a control for their study it was found that BAPN had a previously unreported preference for LOXL2 and LOXL3 over the other members of the LOX family.^[23] Disulfiram (**Figure 5**) a pan-LOX inhibitor with greater potency than thiram was selected as the control molecule rather than using BAPN.^[23]

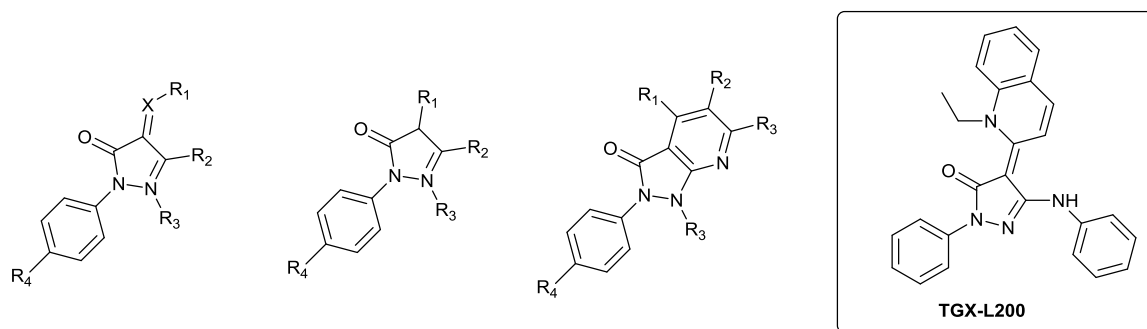


Figure 8. Clusters identified and TGX-L200.^[23]

Ten compounds were identified with a range of selectivity profiles for the individual members of the LOX family. Three of these showed promising LOXL2 selectivity. The three clusters identified are shown in **Figure 8**.^[23] The most potent and selective molecule identified was TGX-L200 (**Figure 8**) with an IC_{50} of 1.0 μ M for LOXL2.^[23]

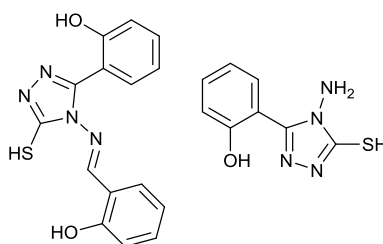


Figure 9. Chemical structures of the triazole derivative inhibitors for LOXL2.^[146]

Muhammad and colleagues designed two triazole derivatives (**Figure 9**) which have promising ΔG binding affinity to LOXL2 using *in silico* methods (-6.2 to -8.9 kcal mol⁻¹).^[146] Both compounds had a stabilised interaction with the active site of LOXL2 *via* the formation of hydrogen bonds and π -stacking interactions. Unfortunately, the crystal structure of LOXL2 was not published at the time of publication and therefore a predicted model of LOXL2 was used in this study.^[146] No biological testing to confirm the *in silico* results was reported.

To date, the majority of small molecule LOXL2 inhibitors reported contain a primary amine motif. This is an important motif that has been exploited to compete with lysine in the direct interaction and reaction with the LTQ cofactor in the active site of LOXL2 (**Scheme 2**) and allows for specific binding to the protein.

5. Conclusions

In summary, LOXL2 is involved in cellular processes such as activation of fibroblasts and promotion of continuous cell growth. This can lead to cancer and fibrosis, diseases with limited therapeutic options available at present.

Overall, LOXL2 is a viable drug target owing to its activity both intracellularly and extracellularly, in addition to its reported effects in facilitating the process of angiogenesis. LOXL2 has also been implicated to be important in MET, the reverse phase of EMT. This process is essential in the final stage of metastasis and colonization, therefore targeting LOXL2 is an attractive target as its role is specific to cancer cells, and not normal cells.

Despite poor outcomes in clinical trials with antibodies, (Simtuzumab has progressed into phase II clinical trials with limited success in improving disease free progression), the use of next generation small molecule LOXL2 inhibitors may be most effectively used as a personalised medicine in patients who have high LOXL2 expression or poor treatment options, for instance, TNBC and RCC patients.

Cross-talk between cancer cells and the environment is essential for tumour progression. LOXL2 plays a role in cancer metastasis in both these aspects, therefore, remains a target of high interest. Although reports implicate LOXL2 to be involved in a number of diverse mechanisms contributing to metastatic potential, this may also be beneficial from a therapeutic aspect; anti-LOXL2 agents would have the potential to inhibit a multitude of pathways involved in tumour progression in addition to uncovering a greater understanding of signal transduction leading to metastasis.

As LOXL2 expression has shown to be erratic in cell lines, the identification of LOXL2 expression in a broader set of cancer cell lines would be beneficial to facilitate this field of research; discovering which cells have LOXL2 sensitivity. Although cellular studies are not the preferred model of metastasis, once identified, these cell lines can then be used for transfection of animal models, which will provide a greater representation of anti-LOXL2 therapy in prospective clinical trials as a personalised medicine.

Additionally, there is an increasing need for a broader range of *in vivo* studies in models other than breast cancer to analyse combination therapy of anti-LOXL2 agents with chemotherapy regimens. Immunohistochemistry of anti-LOXL2 agents in these models would also identify the subcellular location the agent is acting on, ensuring it is effectively targeting LOXL2.

In this review, it is evident that a more conclusive outcome on the role of LOXL2 in metastasis is urgently required which can be achieved *via* the identification of alternative anti-LOXL2 agents, such as small molecule inhibitors. The application of these agents in pre-clinical trials is the next step forward: PAT-1251 is the first small molecule inhibitor against LOXL2 which has entered clinical testing and PXS-2SA has undergone pre-clinical testing with promising early results in cancer therapy.

Small molecule LOXL2 inhibitors are at a much earlier stage of development compared to the antibody approach, and at the time of writing the results from all the clinical trials are not available but with a greater understanding of the target and detailed pharmacokinetic studies, it is the hope that a new therapy from within the compounds discussed in this review can be identified for patient benefit.

Acknowledgements

The authors thank the School of Pharmacy for funding this project.

† V. Chopra and R. M. Sangarapillai contributed equally to this work.

Received: ((will be filled in by the editorial staff))

Revised: ((will be filled in by the editorial staff))

Published online: ((will be filled in by the editorial staff))

References

- [1] Cancer Research UK, *Cancer Statistics for the UK*, <https://www.cancerresearchuk.org/health-professional/cancer-statistics-for-the-uk#heading-Two>, accessed: August, **2019**.
- [2] S. Norouzi, M. V. Gorgi, F. Mosaffa, M. R. Zirak, P. Zamani, J. Behravan, *Crit. Rev. Oncol. Haematol.* **2018**, *132*, 145.
- [3] G. Disibio, S.W. French. *Arch. Pathol. Lab. Med.* **2008**, *132*, 931.
- [4] National Cancer Institute, *Metastatic Cancer*, <https://www.cancer.gov/types/metastatic-cancer>, accessed: August, **2019**.
- [5] R. Daneman, A. Prat, A. *Cold Spring Harb. Perspect. Biol.* **2015**, *7*, 1.
- [6] D. Sarrio, S. M. Rodriguez-Pinilla, D. Hardisson, A. Cano, G. Moreno-Bueno, J. Palacios. *Cancer Res.* **2008**, *68*, 989.
- [7] B. D. Craene, G. Berx, G. *Nat. Rev. Canc.* **2013**, *13*, 97.
- [8] G. Berx, F. Van Roy. *Cold Spring Harb. Perspect. Biol.*, **2009**, *1*, a003129.
- [9] I. Muqbil, J. Wu, A. Aboukameel, R. M. Mohammad, A. S. Azmi. *Sem. Cancer Biol.* **2014**, *27*, 39.
- [10] M. A. Nieto. *Science* **2013**, *342*, 1234850.
- [11] M. A. Nieto. *Cell* **2016**, *166*, 21-45.
- [12] C. L. Chaffer, B. P. San Juan, R. A. Weinberg. *Cancer Metastasis Rev.* **2016**, *35*, 645-654.
- [13] A. Dongre, R. A. Weinberg. *Nat. Rev. Mol. Cell. Biol.* **2019**, *20*, 69-84.
- [14] R. M. Bremnes, T. Dønnem, S. Al-Saad, K. Al-Shibli, S. Andersen, R. Sirera, C. Camps, I. Martinez, L. Busund. *J. Thorac. Oncol.*, **2011**, *6*, 209.
- [15] Y. Katsuno, S. Lamouille, R. Derynck, *Curr. Opin. Oncol.* **2013**, *25*, 76.
- [16] J. Heuberger, W. Birchmeier. *Cold Spring Harb. Perspect. Biol.*, **2010**, *2*, a002915.
- [17] H.-J. Moon, J. Finney, T. Ronnebaum, M. Mure, *Bioorg. Chem.* **2014**, *57*, 231-241.
- [18] L. I. Smith-Mungo, H. M. Kagan, *Matrix Biol.* **1998**, *16*, 387-398.
- [19] L. Wu, Y. Zhu. *Int. J. Mol. Med.*, **2015**, *36*, 1200.
- [20] J. H. Hutchinson, M. W. Rowbottom, D. Lonergan, J. Darlington. P. Prodanovich, C. D. King, J. F. Evans, G. Bain. *ACS Med. Chem. Lett.*, **2017**, *8*, 423.
- [21] E. Hohenester, T. Sasaki, R. Timpl. *Nat. Struct. Biol.*, **1999**, *6*, 228.
- [22] X. Zhang, Q. Wang, J. Wu, J. Wan, Y. Shi, M. Liu. *Proc. Natl Acad. Sci. USA.* **2018**, *115*, 3828.
- [23] I. Hajdú, J. Kardos, B. Major, G. Fabó, Z. Lőrincz, S. Cseh, G. Dorman. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 3113.
- [24] S. D. Vallet, M. Guérilot, N. Belloy, M. Dauchez, S. Ricard-Blum, *ACS Omega*, **2019**, *4*, 8495-8505.
- [25] S. D. Vallet, S. Ricard-Blum, *Essays Biochem.* **2019**, doi: 10.1042/EBC20180050.

- [26] C. E. H. Schmelzer, A. Heinz, H. Troilo, M. P. Lockhart-Cairns, T. A. Jowitt, M. F. Marchand, L. Bidault, M. Bignon, T. Hedtke, A. Barret, J. C. McConnell, M. J. Sherratt, S. Germain, D. J. S. Hulmes, C. Baldock, L. Muller, *FASEB J.* **2019**, *33*, 5468-5481.
- [27] S. X. Wang, M. Mure, K. F. Medzihradsky, A. L. Burlingame, D. E. Brown, D. M. Dooley, A. J. Smith, H. M. Kagan, J. P. Klinman, *Science*, **1996**, *273*, 1078–1084.
- [28] M. Mure, S.X. Wang, J.P. Klinman, *J. Am. Chem. Soc.*, **2003**, *125*, 6113–6125.
- [29] S. L. Payne, B. Fogelgren, A. R. Hess, E. A. Seftor, E. L. Wiley, S. F. T. Fong, K. Csiszar, M. J. C. Hendrix, D. A. Kirschmann. *Cancer Res.*, **2005**, *65*, 11429.
- [30] V. Barry-Hamilton, R. Spangler, D. Marshall, S. McCauley, H. M. Rodriguez, M. Oyasu, A. Mikels, M. Vaysberg, H. Ghermazien, C. Wai, C. A. Garcia, A. C. Velayo, B. Jorgensen, D. Biermann, D. Tsai, J. Green, S. Zaffryar-Eilot, A. Holzer, S. Ogg, D. Thai, G. Neufeld, P. Van Vlasselaer, V. Smith. *Nat. Med.*, **2010**, *16*, 1009.
- [31] M. Janyasupab, Y. Lee, Y. Zhang, C. W. Liu, J. Cai, A. Popa, A. C. Samia, K. W. Wang, J. Xu, C. Hu, M. K. Wendt, B. J. Schiemann, C. L. Thompson, Y. Yen, W. P. Schiemann, C. C. Liu. *Recent Pat. Biomarkers*, **2016**, *5*, 93.
- [32] A. J. Lopez-Jimenez, T. Basak, R. M. Vanacore. *J. Biol. Chem.* **2017**, doi: 10.1074/jbc.M117.798603.
- [33] K. Odaka, H. J. Moon, J. Finney, A. Meier, M. Mure. *Biochemistry* **2018**, *57*, 6973-6983.
- [34] G. Akiri, E. Sabo, H. Dafni, Z. Vadasz, Y. Kartvelishvily, N. Gan, O. Kessler, T. Cohen, M. Resnick, M. Neeman, G. Neufeld, *Cancer Res.* **2003**, *63*, 1657–1666.
- [35] P. Hollosi, J.K. Yakushiji, K.S. Fong, K. Csiszar, S.F. Fong, *Int. J. Cancer*, **2009**, *125*, 318–327.
- [36] G. Moreno-Bueno, F. Salvador, A. Martín, A. Floristán, E. P. Cuevas, V. Santos, A. Montes, S. Morales, M. A. Castilla, A. Rojo-Sebastián, A. Martínez, D. Hardisson, K. Csiszar, F. Portillo, H. Peinado, J. Palacios, A. Cano. *EMBO Mol. Med.* **2011**, *3*, 528-544.
- [37] G. Canesin, E. P. Cuevas, V. Santos, C. López-Menéndez, G. Moreno-Bueno, Y. Huang, K. Csiszar, F. Portillo, H. Peinado, D. Lyden, A. Cano. *Oncogene* **2015**, *34*, 951-964.
- [38] F. Salvador, A. Martin, C. López-Menéndez, G. Moreno-Bueno, V. Santos, A. Vázquez-Naharro, P. G. Santamaria, S. Morales, P. R. Dubus, L. Muninelo-Romay, R. López- López, J. C. Tung, V. M. Weaver, F. Portillo, A. Cano. *Cancer Res.* **2017**, *77*, 5846-5859.
- [39] H. Peinado, M. Del Carmen Iglesias-de la Cruz, D. Olmeda, K. Csiszar, K. S. K. Fong, S. Vega, M. A. Nieto, A. Cano, F. Portillo. *EMBO J.* **2005**, *24*, 3446-3458.
- [40] E. P. Cuevas, G. Moreno-Bueno, G. Canesin, V. Santos, F. Portillo, A. Cano. *Biol. Open.* **2014**, *3*, 129-137.
- [41] M. W. Rowbottom, G. Bain, I. Calderon, T. Lasof, D. Lonergan, A. Lai, F. Huang, J. Darlington, P. Prodanovich, A. M. Santini, C. D. King, L. Goulet, K. E. Shannon, G. L. Ma, K. Nguyen, D. A. MacKenna, J. F. Evans, J. H. Hutchinson. *J. Med. Chem.* **2017**, *60*, 4403.
- [42] N. Herranz, N. Dave, A. Millanes-Romero, L. Pascual-Reguant, L. Morey, V. M. Díaz, V. Lórenz-Fonfría, R. Gutierrez-Gallego, C. Jerónimo, A. Iturbide, L. Di Croce, A. García de Herreros, S. Peiró. *FEBS J.*, **2016**, *283*, 4263.
- [43] A. Iturbide, A. García de Herreros, S. Peiró. *FEBS J.*, **2014**, *282*, 1768.
- [44] L. Peng, Y. Ran, H. Hu, L. Yu, Q. Liu, Z. Zhou, Y. Sun, L. Sun, J. Pan, L. Sun, P. Zhao, Z. Yang. *Carcinogenesis*, **2009**, *30*, 1660.
- [45] A. Tomar, D. David. *Curr. Opin. Cell Biol.*, **2009**, *21*, 676.
- [46] N. Ikenaga Z. W. Peng, K. A. Vaid, S. B. Liu, S. Yoshida, D. Y. Sverdlov, A. Mikels-Vigdal, V. Smith, D. Schuppan, Y. V. Popov. *Gut*, **2017**, *66*, 1697.
- [47] A. Puente, J. I. Fortea, J. Cabezas, M. T. A. Loste, P. Iruzubieta, S. Llerena, P. Huelin, E. Fábrega, J. Crespo, *Int. J. Mol. Sci.* **2019**, *20*, 1634.
- [48] S. Stangenberg S. Saad, H. C. Schilter, A. Zaky, A. Gill, C. A. Pollock, M. G. Wong. *Sci. Rep.* **2018**, *8*, 9423.
- [49] D. Saxena, F. Mahjour, A. D. Findlay, E. A. Mously, A. Kantarci, P. C. Trackman. *J. Dent. Res.*, **2018**, *97*, 1277.
- [50] V. Aumiller, B. Strobel, M. Romeike, M. Schuler, B. E. Stierstorfer, S. Kreuz. *Sci. Rep.*, **2017**, *7*, 149.
- [51] D. A. Kirschmann, E. A. Seftor, S. F. T. Fong, D. R. C. Nieva, C. M. Sullivan, E. M. Edwards, P. Sommer, K. Csiszar, M. J. C. Hendrix. *Cancer Res.* **2002**, *62*, 4478.

- [52] T. Van Bergen, D. Marshall, S. Van de Veire, E. Vandewalle, L. Moons, J. Herman, V. Smith, I. Stalmans. *Invest. Ophthalmol. Vis. Sci.* **2013**, *54*, 5788.
- [53] J. Yang, K. Savvatis, J. S. Kang, P. Fan, H. Zhong, K. Schwartz, V. Barry, A. Mikels-Vigdal, S. Karpinski, D. Kornyejev, J. Adamkewicz, X. Feng, Q. Zhou, C. Shang, P. Kumar, D. Phan, M. Kasner, B. López, J. Diez, K. C. Wright, R. L. Kovacs, P.-S. Chen, T. Quertermous, V. Smith, L. Yao, C. Tschöpe, C.-P. Chang. *Nat. Commun.*, **2016**, *7*, 13710.
- [54] S. Zaffryar-Eilot, D. Marshall, T. Voloshin, A. Bar-Zion, R. Spangler, O. Kessler, H. Ghermazien, V. Brekman, E. Suss-Toby, D. Adam, Y. Shaked, V. Smith, G. Neufeld. *Carcinogenesis*, **2013**, *34*, 2370.
- [55] H. E. Barker, J. Chang, T. R. Cox, G. Lang, D. Bird, M. Nicolau, H. R. Evans, A. Gartland, J. T. Erler. *Cancer Res.* **2011**, *71*, 1561.
- [56] H. E. Barker, D. Bird, G. Lang, J. T. Erler. *Mol. Cancer Res.*, **2013**, *11*, 1425.
- [57] A. B. Benson, Z. A. Wainberg, J. R. Hecht, D. Vyushkov, H. Dong, J. Bendell, F. Kudrik. *Oncologist*, **2017**, *22*, 241-e15.
- [58] J. R. Hecht, A. B. Benson, D. Vyushkov, Y. Yang, J. Bendell, U. Verma, U. *Oncologist*, **2017**, *22*, e23.
- [59] G. Raghu, K. K. Brown, H. R. Collard, V. Cottin, K. F. Gibson, R. J. Kaner, D. J. Lederer, F. J. Martinez, P. W. Noble, J. W. Song, A. U. Wells, T. P. Whelan, W. Wuyts, E. Moreau, S. D. Patterson, V. Smith, S. Bayly, J. W. Chien, Q. Gong, J. J. Zhang, T. G. O'Riordan, *Lancet Respir. Med.* **2017**, *5*, 22.
- [60] A. J. Muir, C. Levy, H. L. A. Janssen, A. J. Montano-Loza, M. L. Shiffman, S. Caldwell, V. Luketic, D. Ding, C. Jia, B. J. McColgan, J. G. McHutchison, G. M. Subramanian, R. P. Myers, M. Manns, R. Chapman, N. H. Afdhal, Z. Goodman, B. Eksteen, C. L. Bowlus, and GS-US-321-0102 Investigators, *Hepatology*, **2018**, *69*, 684.
- [61] S. Verstovsek, M. R. Savona, R. A. Mesa, H. Dong, J. D. Maltzman, S. Sharma, J. Silverman, S. T. Oh, J. Gotlib. *Br. J. Haematol.*, **2017**, *176*, 939.
- [62] S. A. Harrison, M. F. Abdelmalek, S. Caldwell, M. L. Shiffman, A. M. Diehl, R. Ghalib, E. J. Lawitz, D. C. Rockey, R. A. Schall, C. Jia, B. J. McColgan, J. G. McHutchison, G. M. Subramanian, R. P. Myers, Z. Younossi, V. Ratziu, A. J. Muir, N. H. Afdhal, Z. Goodman, J. Bosch, A. J. Sanyal, GS-US-321-0105 and GS-US-321-0106 Investigators. *Gastroenterology*, **2018**, *155*, 1140.
- [63] S. A. Townsend, P. N. Newsome. *Br. Med. Bull.*, **2016**, *119*, 143.
- [64] Gilead Sciences, Safety and Efficacy of Simtuzumab (GS-6624) in Adults With Advanced Liver Fibrosis But Not Cirrhosis Secondary to Non-Alcoholic Steatohepatitis (NASH), <https://clinicaltrials.gov/ct2/show/NCT01672866>, accessed: August **2019**.
- [65] Gilead Sciences, Simtuzumab (GS-6624) in the Treatment of Cirrhosis Due to NASH (NASH), <https://clinicaltrials.gov/ct2/show/NCT01672879>, accessed: August **2019**.
- [66] P. LoRusso, J. R. Hecht, D. L. Thai, M. J. Hawkins, H. Dong, A. W. Tolcher. *J. Clin. Oncol.*, **2014**, *32*, 554.
- [67] F. Rückert, P. Joensuu, H.-D. Saeger, R. Grützmann, C. Pilarsky. *Int. J. Colorectal Dis.*, **2010**, *25*, 303.
- [68] Gilead Sciences, First-in-human Study of AB0024 to Evaluate Safety and Tolerability in Adults with Advanced Solid Tumors, <https://clinicaltrials.gov/ct2/show/NCT01323933>, accessed: August **2019**.
- [69] H. M. Rodriguez, M. Vaysberg, A. Mikels, S. McCauley, A. C. Velayo, C. Garcia, V. Smith. *J. Biol. Chem.* **2010**, 20964.
- [70] J. S. Park, J. Lee, Y. S. Lee, J. K. Kim, S. M. Dong, D. S. Yoon. *Oncotarget*, **2016**, *7*, 42539.
- [71] A. I. Phipps, D. D. Buchanan, K. W. Makar, A. K. Win, J. A. Baron, N. M. Lindor, J. D. Potter, P. A. Newcomb. *Br. J. Cancer*, **2013**, *108*, 1757.
- [72] P. Park, S. J. Jo, M. J. Kim, H. J. Kim, J. H. Lee, C. K. Park, H. Kim, K. Y. Lee, H. Kim, J. H. Park, S. M. Dong, J. M. Lee. *Oncotarget*, **2017**, *8*, 80325.
- [73] P. Fickert. *Hepatology* **2019**, *69*, 476-479.
- [74] H. E. Barker, J. T. Erler, *Future Oncol.* **2011**, *7*, 707-710.
- [75] M. Grossman, N. Ben-Chetrit, A. Zhuravlev, R. Afik, E. Bassat, I. Solomonov, Y. Yarden, I. Sagi. *Cancer Res.*, **2016**, *76*, 4249-4258.

- [76] (a) S. Ahn, S. Dong, A. Oshima, W. Kim, H. Lee, S. Lee, S. Kwon, J. Lee, J. Lee, J. Jeong, H. Lee, J. Green. *Breast Cancer Res. Treat.* **2013**, *141*, 89; (b) S. G. Ahn, S. J. Kim, C. Kim, J. Jeong, *J. Breast Canc.* **2016**, *19*, 223.
- [77] J. P. Cebrià-Costa, L. Pascual-Reguant, A. Gonzalez-Perez, G. Serra-Bardenys, J. Querol, M. Cosín, G. Verde, R. A. Cigliano, W. Sanserverino, S. Seguar-Bayona, A. Iturbide, D. Andreu, P. Nuciforo, C. Bernando-Morales, V. Rodilla, J. Arribas, J. Yelamos, A. Garcia de Herreros, T. H. Stracker, S. Peiró, *Oncogene*, **2019**, doi: 10.1038/s41388-019-0969-1.
- [78] T. Li, L. Xu, Z. Wu, L. Liao, J. Shen, X. Xu, Z. Du, Q. Zhao, E. Li. *Hum. Pathol.* **2012**, *43*, 1068.
- [79] X. Zhan, J. Jiao, H. Zhang, C. Li, J. Zhao, L. Liao, J. Wu, B. Wu, Z. Wu, S. Wang, Z. Du, J. Shen, H. Zou, G. Neufeld, L. Xu, E. Li. *Cancer Med.* **2017**, *6*, 1707.
- [80] A. Fantozzi, G. Christofori. *Breast Cancer Res.* **2006**, *8*, 212.
- [81] J. Kitz, L. E. Lowes, D. Goodale, A. L. Allan. *Diagnostics*, **2018**, *8*, 30.
- [82] H. Moon, J. Finney, L. Xu, D. Moore, D. R. Welch, M. Mure. *J. Biol. Chem.* **2013**, *288*, 30000.
- [83] S. Wu, Q. Zheng, X. Xing, Y. Dong, Y. Wang, Y. You, R. Chen, C. Hu, J. Chen, D. Gao, Y. Zhao, Z. Wang, T. Xue, Z. Ren, J. Cui. *J. Exp. Clin. Cancer Res.* **2018**, *37*, 99.
- [84] Pancreatic Cancer Action, UK Pancreatic Cancer Prognosis and Survival Rates, <https://pancreaticcanceraction.org/about-pancreatic-cancer/medical-professionals/stats-facts/prognosis-survival/>, accessed: August **2019**.
- [85] Cancer Research UK, Bowel Cancer Statistics, <https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer#heading-Zero>, accessed: August, **2019**.
- [86] D. Dinu, M. Dobre, E. Panaitescu, R. Bîrlă, C. Iosif, P. Hoara, A. Caragui, M. Boeriu, S. Constantinoiu, C. Ardeleanu. *J. Med. Life*, **2014**, *7*, 581.
- [87] Cancer Research UK, *Oesophageal cancer survival statistic*, <https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/oesophageal-cancer/survival#heading-Zero>, accessed: August, **2019**.
- [88] Y. Zhu, M. Zhu, X. Zhang, X. Xu, Z. Wu, L. Liao, L. Li, Y. Xie, J. Wu, H. Zou, J. Xie, E. Li, L. Xu. *Hum. Pathol.* **2016**, *52*, 153.
- [89] B.-L. Wu, G.-Q. Lv, H.-Y. Zou, Z.-P. Du, J.-Y. Wu, P.-X. Zhang, L.-Y. Xu, E.-M. Li, *Sci. World J.* **2014**, 431792.
- [90] G.-Q. Lv, H.-Y. Zou, L.-D. Liao, H.-H. Cao, F.-M. Zeng, B.-L. Wu, J.-J. Xie, W.-K. Fang, L.-Y. Xu, E.-M. Li, *Biochem. Cell Biol.* **2014**, *92*, 379.
- [91] H. Peinado, G. Moreno-Bueno, D. Hardisson, E. Pérez-Gómez, V. Santos, M. Mendiola, J. I. De Diego, M. Nistal, M. Qunitanilla, F. Portillo, A. Cano. *Cancer Res.* **2008**, *68*, 4541-4550.
- [92] A. Martin, F. Salvador, G. Moreno-Bueno, A. Floristán, C. Ruiz-Heguido, E. P. Cuevas, S. Morales, V. Santos, K. Csiszar, P. Dubus, J. J. Haigh, A. Bigas, F. Portillo, A. Cano. *EMBO J.* **2015**, *34*, 1090-1109.
- [93] F. Mahjour, V. Dambal, N. Shrestha, V. Singh, V. Noonan, A. Kantarci, P. C. Trackman, *Oncogenesis*, **2019**, *8*, 34.
- [94] H. Kasashima, M. Yashiro, H. Kinoshita, T. Fukuoka, T. Morisaki, K. Sakurai, N. Kubo, M. Ohira, K. Hirakawa. *Cancer Lett.* **2014**, *354*, 438.
- [95] Cancer Research U.K, *Lung cancer statistics*, <https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/lung-cancer#heading-One>, accessed: August, **2019**.
- [96] P. Zhan, X. Lv, Y. Ji, H. Xie, L. Yu. *Clin. Respir. J.*, **2018**, *12*, 712.
- [97] K. Mizuno, N. Seki, H. Mataka, R. Matsushita, K. Kamikawaji, T. Kumamoto, K. Takagi, Y. Goto, R. Nishikawa, M. Kato, H. Enokida, M. Nakagawa, H. Inoue. *Int. J. Oncol.*, **2016**, *48*, 450.
- [98] M. Ye, J. Zhang, T. Guo, X. Pan. *Biomed. Pharmacother.* **2018**, *97*, 1289.
- [99] R. Nishikawa, T. Chiyomaru, H. Enokida, S. Inoguchi, T. Ishihara, R. Matsushita, Y. Goto, I. Fukumoto, M. Nakagawa, N. Seki. *FEBS Lett.*, **2015**, *589*, 2136.
- [100] A. Kurozumi, M. Kato, Y. Goto, R. Matsushita, R. Nishikawa, A. Okato, I. Fukumoto, T. Ichikawa, N. Seki. *Int. J. Oncol.*, **2016**, *48*, 1837.
- [101] D. H. Peng, C. Ungewiss, P. Tong, L. A. Byers, J. Wang, J. R. Canales, P. A. Villalobos, N. Uraoka, B. Mino, C. Behrens, I. I. Wistuba, R. I. Han, C. A. Wana, M. Fahrenholtz, K. J. Grandallen, C. J. Creighton, D. L. Gibbons. *Oncogene*, **2017**, *36*, 1925.

- [102] Y. Qu, H. Xiao, W. Xiao, Z. Xiong, W. Hu, Y. Gao, Z. Ru, C. Wang, L. Bao, K. Wang, H. Ruan, Z. Song, K. Chen, X. Zhang, H. Yang. *Cell. Physiol. Biochem.*, **2018**, *48*, 1075.
- [103] D. H. Craighead, H. Wang, L. Santhanam, L. M. Alexander. *Am. J. Physiol. Circ. Physiol.*, **2018**, *314*, H424.
- [104] C. Wiel, A. Augert, D. F. Vincent, D. Gitenay, D. Vindrieux, B. Le Calvé, V. Arfi, H. Lallet-Daher, C. Reynaud, I. Treilleux, L. Bartholin, E. Lelievre, D. Bernard. *Cell Death Dis.*, **2013**, *4*, e855.
- [105] M. Nilsson, H. Adamo, A. Bergh, S. Halin Bergström. *Sci. Rep.* **2016**, *6*, 19608.
- [106] G. Tjin, E. S. White, A. Faiz, D. Sicard, D. J. Tschumperlin, A. Mahar, E. P. W. Kable, J. K. Burgess. *Dis. Model Mech.*, **2017**, *10*, 1301.
- [107] A. H. Nave, I. Mizikova, G. Niess, H. Steenbock, F. Reichenberger, M. L. Talavera, F. Veit, S. Herold, K. Mayer, I. Vadász, N. Weissmann, W. Seeger, J. Brinckmann, R. E. Morty. *Arterioscler. Thromb. Vasc. Biol.*, **2014**, *34*, 1446.
- [108] C. C.-L. Wong, H. Zhang, D. M. Gilkes, J. Chen, H. Wei, P. Chaturvedi, M. E. Hubbi, G. L. Semenza. *J. Mol. Med.* **2012**, *90*, 803.
- [109] Y.-M. Kim, E.-C. Kim, Y. Kim. *Mol. Biol. Rep.*, **2011**, *38*, 145.
- [110] S. T. Jung, M. S. Kim, J. Y. Seo, H. C. Kim, Y. Kim. *Protein Expr. Purif.*, **2003**, *31*, 240.
- [111] Z. Vadasz, O. Kessler, G. Akiri, S. Gengrinovitch, H. M. Kagan, Y. Baruch, O. B. Izhak, G. Neufeld. *J. Hepatol.*, **2005**, *43*, 499.
- [112] J. Liu, J. Luan, X. Zhou, Y. Cui, J. Han. *Intractable Rare Dis. Res.*, **2017**, *6*, 249.
- [113] I. Mižiková, F. Palumbo, T. Tábi, S. Herold, I. Vadász, K. Mayer, W. Seeger, R. E. Morty. *Physiol. Genomics*, **2017**, *49*, 416.
- [114] A. J. Coffey, M. Durkie, S. Hague, K. McLay, J. Emmerson, C. Lo, S. Klaffke, C.J. Joyce, A. Dhawan, N. Hadzic, G. Mieli-Vergani, R. Kirk, K. Elizabeth Allen, D. Nicholl, S. Wong, W. Griffiths, S. Smithson, N. Giffin, A. Taha, S. Connolly, G. T. Gillett, S. Tanner, J. Bonham, B. Sharrack, A. Palotie, M. Rattray, A. Dalton, O. Bandmann. *Brain*, **2013**, *136*, 1476.
- [115] D. Cosgrove, B. Dufek, D. T. Meehan, D. Delimont, M. Hartnett, G. Samuelson, M. A. Gratton, G. Phillips, D. A. MacKenna, G. Bain. *Kidney Int.*, **2018**, *94*, 303.
- [116] J. Lugassy, S. Zaffryar-Eilot, S. Soueid, A. Mordoviz, V. Smith, O. Kessler, G. Neufeld. *J. Biol. Chem.*, **2012**, *287*, 3541.
- [117] D. A. Abourbih, S. Di Cesare, M. E. Orellana, E. Anteck, C. Martins, L. A. Petrucci, M. N. Burnier Jr. *Melanoma Res.*, **2010**, *20*, 97.
- [118] G. Ninomiya, S. Yamada, M. Hayashi, S. Takeda, M. Suenaga, H. Takami, M. Kanda, N. Iwata, Y. Niwa, C. Tanaka, D. Kobayashi, T. Fujii, G. Nakayama, H. Sugimoto, M. Koike, M. Fujiwara, Y. Koda. *Oncol. Rep.*, **2018**, *39*, 2664.
- [119] J. Chang, M. C. Lucas, L. E. Leonte, M. Garcia-Montolio, L. B. Singh, A. D. Findlay, M. Deodhar, J. S. Foot, W. Jarolim, P. Timpson, J.T. Erler, T. R. Cox. *Oncotarget*, **2017**, *8*, 26066.
- [120] Y. Wang, X. Xu, P. Zhao, B. Tong, Z. Wei, Y. Dai. *Oncotarget*, **2016**, *7*, 23684.
- [121] N. Segond, S. A. Degrelle, S. Berndt, E. Clouqueur, C. Rouault, B. Saubamea, P. Dessen, K. S. Fong, K. Csiszar, J. Badet, D. Evain-Brion, T. Fournier. *PLoS One*, **2013**, *8*, e79413.
- [122] L. Dudakova, P. Liskova, T. Trojek, M. Palos, S. Kalasova, K. Jirsova. *Exp. Eye Res.*, **2012**, *104*, 74.
- [123] U. Schlötzer-Schrehardt, C. M. Hammer, A. W. Krysta, C. Hofmann-Rummelt, F. Pasutto, T. Sasaki, F. E. Kruse, M. Zenkel. *Ophthalmology*, **2012**, *119*, 1832.
- [124] A. Sethi, W. Mao, R. J. Wordinger, A. F. Clark. *Investig. Ophthalmology Vis. Sci.*, **2011**, *52*, 5240.
- [125] D. C. Twedt, in *Canine and Feline Gastroenterology*, Vol. 1 (Ed. R. J. Washabau, M. J. Day), Elsevier Saunders, St. Louis, USA **2013**, 489.
- [126] J. A. Tumlin. *WO2016094415*, **2016**.
- [127] P. Netter, B. Bannwarth, P. Pere, A. Nicolas. *Clin. Pharmacokinet.* **1987**, *13*, 317.
- [128] A. Mayorca-Guiliani, J. T. Erler. *Oncol. Targets Ther.*, **2013**, *6*, 1729.
- [129] R. C. Siegel. *J. Biol. Chem.* **1977**, *252*, 254.
- [130] M. J. Pollheimer, S. Racedo, A. Mikels-Vigdal, D. Marshall, C. Bowlus, C. Lackner, T. Madl, T. H. Karlsen, J. R. Hov, S. K. Lyman, J. Adamkiewicz, V. Smith, E. Moreau, G. Zollner, T. J. Eide, T. Stojakovic, H. Scharnagl, H. J. Gruber, R. E. Stauber, M. Trauner, P. Fickert. *J. Hepatol.* **2018**, *69*, 368.

- [131] N. F. Larusso, R. H. Wiesner, J. Ludwig, R. L. Maccarty, S. J. Beaver, A. R. Zinsmeister. *Gastroenterology*, **1988**, 95, 1036.
- [132] H. Ovet, F. Oztay. *Biol. Trace Elem. Res.*, **2014**, 162, 189.
- [133] M. W. Rowbottom, J. H. Hutchinson, J. Howard. *WO2017015221*, **2017**,
- [134] M. W. Rowbottom, J. H. Hutchinson, D. Lonergan. *WO2018048930*, **2018**.
- [135] A. D. Findlay, C. I. Turner, M. Deodhar, J. S. Foot, W. Jarolimek, W. Zhou, A. D. Robertson., *et al.* *WO2017136871*, **2017**.
- [136] L. Leung, D. Niculescu-Duvaz, D. Smithen, F. Lopes, C. Callens, R. McLeary, G. Saturno, L. Davies, M. Aljarah, M. Brown, L. Johnson, A. Zambon, T. Chambers, D. Menard, N. Bayliss, R. Knight, L. Fish, R. Lawrence, M. Challinor, H. Tang, R. Marais, C. Springer, *J. Med. Chem.* **2019**, 62, 5863-5884.
- [137] C. Springer, R. Marais, D. Niculescu-Duvaz, L. Leung, D. Smithen, C. Callens, H. Tang. *WO2017141049*, **2017**.
- [138] H. Tang, L. Leung, G. Saturno, A. Viros, D. Smith, G. Di Leva, E. Morrison, D. Niculescu-Duvaz, F. Lopes, L. Johnson, N. Dhomen, C. Springer, R. Marais, *Nat. Commun.* **2017**, 8, 14909.
- [139] D. A. Smithen, L. M. H. Leung, M. Challinor, R. Lawrence, H. Tang, D. Niculescu-Duvaz, S. P. Pearce, R. Mcleary, F. Lopes, M. Aljarah, M. Brown, L. Johnson, G. Thomson, R. Marais, C. Springer. *J. Med. Chem.* **2019**, <https://doi.org/10.1021/acs.jmedchem.9b01112>.
- [140] A. D. Findlay, C. I. Turner, M. Deodhar, J. S. Foot, W. Zhou, W. Jarolimek, A. D. Robertson. *WO2018157190*, **2018**.
- [141] R. Marais, C. Springer, D. Niculescu-Duvaz, N. Miller, M. Aljarah, A. Zambon, L. Leung, D. Smithen, M. Brown, T. Haoran, PCT WO 2019/073251, **2019**.
- [142] Pharmaxis, Pharmaxis Releases Positive Results of Phase 1 Clinical Trial for Second LOXL2 Inhibitor Compound, <http://www.pharmaxis.com.au/investor-centre/news/view/pharmaxis-releases-positive-results-of-phase-1-clinical-trial-for-second-loxl2-inhibitor-compound>, accessed: August, **2019**.
- [143] M. W. Rowbottom J. H., Hutchinson, D. Lonergan. *WO2016144702*, **2016**.
- [144] D. Lonergan, K. R. Holme, M. W. Rowbottom, *WO2018048943*, **2018**.
- [145] PharmAkea, Inc. Single and Multiple Dose Safety, Tolerability, PK and Food Effect Study of PAT1251 in Healthy Adult Subjects, <https://clinicaltrials.gov/ct2/show/study/NCT02852551>, accessed: August, **2019**.
- [146] S. A. Muhammad, A. Ali, T. Ismail, R. Zafar, U. Ilyas, J. Ahmad. *Comput. Biol. Chem.* **2014**, 51, 71.



Vriddhi Chopra received her B.Sc. (Hons) in Biomedical Science from King's College London in 2014 where she completed a research project regarding the regulation of cell motility in metastasis. Vriddhi then returned to university in 2015 to study for an MPharm at the University of Birmingham, completing a research project on LOXL2 biological pathways.



Ruth M. Sangarappillai read for a MPharm at the University of Birmingham from 2015-2019. In 2017, she participated in a research project involving the production and testing of oral mucosal films (supervisor: Professor Ingunn Tho, University of Oslo). For her MPharm dissertation she researched small molecule approaches to LOXL2 inhibition.



Isolda Romero-Canelon's commitment to academic research started at the University of Los Andes, Venezuela where she held a tenure track position before moving to the University of Warwick for her PhD. After completing her Doctoral Thesis she stayed at the University of Warwick as an IAS Early Career Research Fellow, later, as a Postdoctoral Researcher and as a Senior Research Fellow. Isolda is now based at the University of Birmingham as a Lecturer in Medicinal Chemistry and holds a honorary visiting position at the University of Warwick.



Alan M. Jones received his B.Sc. (Hons) from the University of Aberdeen (UK) and his Ph.D. from the University of St Andrews (UK) with Prof. N. J. Westwood. After post-doctoral research in the labs of Prof. S. G. Davies and Dr A. J. Russell (University of Oxford, UK) and Prof. I. Collins (Institute of Cancer Research, UK), he began his independent academic career as a lecturer at Manchester Metropolitan University (UK) in 2014 before moving to his current position at the University of Birmingham (UK) in 2017. His research interests include electrosynthesis applied to medicinal chemistry.

Table of contents entry

Lysyl oxidase-like 2 (LOXL2) is an emerging drug target in oncology with a defined mode of action. A recent dramatic increase in reports of novel small molecule inhibitors warrants a reconsideration of LOXL2 as a drug target. In this review, we summarise the interplay of LOXL2 with cancer and review recent developments in inhibitor development.

Keyword lysyl oxidase like-2 (LOXL2)

Vridhhi Chopra, Ruth M. Sangarappillai, Dr. Isolda Romero-Canelón and Dr. Alan M. Jones*

Lysyl Oxidase Like-2 (LOXL2): An Emerging Oncology Target

