New family of molecules with oral therapeutic potential in NAFLD/NASH, obesity, hypertension, dyslipidemia and type 2 diabetes
Opportunity

- Novel family of small molecules, orally applicable
- **Pre-clinical stage**, lead molecule (SJT4A) identified
- Potential application in a broad variety of metabolic disease indications:
  - **NAFLD (including NASH)**: significant reduction in collagen gene expression (fibrosis marker) and NAS score: reduced liver steatosis, ballooning, inflammation and fibrosis
  - **Type 2 Diabetes**: greater potency compared to standard glucose lowering therapies (Metformin)
  - **Obesity**: > 40% excessive weight reduction in DIO mouse model
  - **Hypertension**: reduces systolic blood pressure
  - **Dyslipidemia and diabetes associated complications**: Decline of insulin resistance, reduction in blood pressure, plasma cholesterol and weight control, decrease of liver lipids in hepatic steatosis
- Encouraging **safety profile** from early toxicology studies
- **Simple manufacturing process**
- “First in Class” novel dual mechanism of action (**FXR agonist and 5-HTR\textsubscript{2A} antagonist**)
- **Strong IP** portfolio with long expiry dates granted in major markets
- SJT is seeking a partner for the further development and commercialization of its proprietary, novel molecules
Discovery pathway to SJT’s novel molecules

IBAMA

Input from local tribe elders

(1) Multiple rounds of testing on plants:
   - Anti-diabetic properties
   - Toxicology
   - Efficacy
(2) Identification of active groups
   (alkaloids, polyphenols & glycosides)
(3) Scaffold selection
   - Addition of radicals
   - Identification of best molecules

AMAZON RAIN FOREST
rich source of medicinal plants

100 plants selected for further analysis (1)

Testing toxicology & efficacy

34 plants selected for anti-diabetic properties (1)

Testing in pairs

2 plants with synergistic activity (1)

Isolate and analyse active principles (2)

Herbal Product

NAFLD/NASH
Obesity
Hypertension
Diabetes Type 2
Dyslipidemia

New family of molecules:
in vivo screening (1 lead and 68 additional active family members)

Animal testing

Parent β-carboline structure

Design and synthesis of new molecules (3)

Synthesized molecule
Company background

• SJT Molecular Research is a privately-funded biotech company based in Spain (Vitoria)
• Focus on the discovery and early development of novel molecules for metabolic disorders
• Virtual set-up with strong network of renowned public and private institutions:

Public Institutions:
• Federal University of Grande Dourados (Brazil)
• Federal University of Paraná (Brazil)
• University of Alcalá (Madrid, Spain)
• University of the Balear Islands (Spain)
• University of Vigo (Spain)

Private Institutions:
• Eurofins, Cerep, Panlabs (France, UK, USA, Taiwan)
• Gubra (Denmark)
• Cyprotex (UK, USA)
• Physiogenex (France)
• Amylgen (France)
• Gentronix (UK)
• Sequani (UK)
• Softmining (IT)
Patent claims cover:
• Composition of matter for the molecules
• Intermediates and derivatives
• Pharmaceutical formulations

Granted in:
• USA, US9440966 (B2)
• Europe, EP2691394 (B1)
• Japan, JP6049216 (B2)
• Canada, 2,831,716
• Australia, AU2012234230 (B2)
• Russia, RU2615136 (C2)
• Israel, 228630
• South Korea, 046182713
• Mexico, MX/a/2013/011124

National phases:
• China
• Brazil

Patent filings worldwide covering novel family of molecules published as WO2012130912.

Novel family of molecules

- Data focused on the **lead** molecule (**SJT4A**)
- 2 backup molecules based on the same structure
- Intermediate and derivat compounds of the 3 molecules covered by patent applications (66 additional molecules)

![Chemical structure of SJT4A](image)
Pharmacokinetics (PK)

- Plasma concentrations high enough to provide a therapeutic effect
- Extended bioavailability by bid administration*

**SJT4A-HCl (mice)**

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>IV 15 mg/kg</th>
<th>PO 50 mg/kg</th>
<th>PO 50 mg/kg</th>
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<tr>
<td></td>
<td>Qdx1**</td>
<td>Qdx7**</td>
<td>Bid*x7</td>
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<tr>
<td>AUC (h x ng/ml)</td>
<td>6229</td>
<td>3988</td>
<td>4836</td>
<td>8132</td>
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<td>Last time point (AUC)</td>
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<tr>
<td>C_0 (ng/ml)</td>
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<tr>
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<tr>
<td>T_{max} (h)</td>
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<td>4.00</td>
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<tr>
<td>CL (ml/min/kg)</td>
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<tr>
<td>Vss (L/kg)</td>
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<td></td>
<td></td>
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<tr>
<td>F (%)</td>
<td></td>
<td>18.87</td>
<td>21.81</td>
<td>35.32</td>
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</tbody>
</table>

* bid: 2 x daily // ** qd: once daily
SJT4A reduces hyperglycemia and hyperinsulinemia

DIO mice model treated with SJT4A for 22 days

Data expressed as mean ± s.e.m. values from 10 animals. *P<0.05, **P<0.01. 4A (50 mg/kg), Metformin (150 mg/kg)
SJT4A decreases insulin resistance from the first week of treatment

Data expressed as mean ± s.e.m. values from 10 animals. *P<0.05, **P<0.01. 4A (50 mg/kg), Metformin (150 mg/kg)
SJT4A decreases liver lipid content associated to hepatic steatosis

DIO mice model treated with SJT4A for 36 days

SJT4A decreases liver lipid content associated to hepatic steatosis

Data expressed as mean ± s.e.m. values from 10 animals. **P<0.01. 4A (50 mg/kg), Metformin (150 mg/kg)
SJT4A reduces the body excess weight (>47 %) and body weight by ≈15 %

Data expressed as mean ± s.e.m. values from 10 animals. **P<0.01, ***P<0.001. 4A (50 mg/kg), Metformin (150 mg/kg)
SJT4A reduces systolic blood pressure

Systolic blood pressure in SHR hypertensive rat model

Data expressed as mean ± s.e.m. values from 6 animals. ***P<0.001. 4A (15 mg/kg)
SJT4A reduces excess weight by >40% and body weight by ≈15%

Data expressed as mean ± s.e.m. values from 10-12 animals. **P<0.01, ***P<0.001. 4A (50 mg/kg)
SJT4A reduces liver excess weight in DIO-NASH mice (>40 %)

Data expressed as mean ± s.e.m. values from 10-12 animals. **P<0.01, ***P<0.001. 4A (50 mg/kg)
SJT4A reduces the excess of TG and TC in liver (>50 %)

Gubra DIO-NASH mice model treated with SJT4A for 8 weeks

**Relative liver TG content**

**Relative liver TC content**

Data expressed as mean ± s.e.m. values from 10-12 animals. **P<0.01, ***P<0.001. 4A (50 mg/kg)
SJT4A decreases liver toxicity

(77 % ALT & 65 % AST)

Gubra DIO-NASH mice model treated with SJT4A for 8 weeks

Data expressed as mean ± s.e.m. values from 10-12 animals. ***P<0.001. 4A (50 mg/kg)
SJT4A significantly lowers NAFLD activity score (NAS)

Gubra DIO-NASH mice model treated with SJT4A for 8 weeks

Significantly lower NAFLD activity score (NAS) affects steatosis and inflammation

Pre and post-study biopsy comparison
SJT4A significantly reduces inflammation

Gubra DIO-NASH mice model treated with SJT4A for 8 weeks

SJT4A reduces inflammation through lowers liver galectin-3 content (inflammation marker)

Data expressed as mean ± s.e.m. values from 10-12 animals. ***P<0.001. 4A (50 mg/kg)
SJT4A decreases fat accumulation in liver

Gubra DIO-NASH mice model treated with SJT4A for 8 weeks

Liver lipid (total) (Post-biopsy)

Data expressed as mean ± s.e.m. values from 10-12 animals. ***P<0.001. 4A (50 mg/kg)
SJT4A reduces total liver collagen type I (fibrosis marker)

Gubra DIO-NASH mice model treated with SJT4A for 8 weeks

Data expressed as mean ± s.e.m. values from 10-12 animals. *P<0.05. 4A (50 mg/kg)
SJT4A significantly reduces collagen gene expression (RNAseq)

Gubra DIO-NASH mice model treated with SJT4A for 8 weeks

Data expressed as mean ± s.e.m. values from 6 animals. ***P<0.001. 4A (50 mg/kg)
**SJT4A contributes to the recovery of dysregulated gene expression in DIO-NASH mice**

Gubra DIO-NASH mice model treated with SJT4A for 8 weeks

- The majority of gene regulated by SJT4A (86.2%) were also differentially expressed between lean-chow and DIO-NASH untreated animals, indicating that SJT4A can mainly affect expression of genes associated with the disease.
- Most of the pathways associated with NASH development have been affected by SJT4A, mainly the hepatic stellate cell (HSC) activation, the central driver of fibrosis in experimental and human liver injury.

**Genes differentially expressed between DIO-NASH mice and lean-chow animals (grey) or DIO-NASH mice treated with SJT4A (blue)**

- Number of genes differentially expressed:
  - LEAN-CHOW Vehicle (BID, PO): 261
  - SJT4a (BID, PO): 1630
  - Total: 5151

**Pathways perturbed**

- Stellate cell activation: 77% LEAN-CHOW, 68% SJT4a
- Monocyte recruitment: 93% LEAN-CHOW, 40% SJT4a
- Hepatocellular cell death: 69% LEAN-CHOW, 15% SJT4a
- Inflammation signaling: 71% LEAN-CHOW, 18% SJT4a
- Insulin signaling: 55% LEAN-CHOW, 15% SJT4a
- Lipid metabolism: 70% LEAN-CHOW, 10% SJT4a
- FXR signaling: 75% LEAN-CHOW, 30% SJT4a
Gene expression regulated by SJT4A stops fibrosis development

Gubra DIO-NASH mice model treated with SJT4A for 8 weeks

Representative genes **up-regulated** in liver of DIO-NASH mice and recovered by treatment with SJT4A:

- Involved in hepatic liver metabolism (steatosis): Cidea, Cicec & Mogat1 (lipid droplet), CD36
- Involved in inflammation and macrophage recruitment: IL members & Rc (1, 17), CCL members (MCP-1)
- Involved in fibrosis:
  - ECM components: collagens, laminins, elastin, fibrillins, fibulins, Efemps, vimentin, cytoglobin, α-SMA
  - Proteoglycans: lumican, decorin, fibromodulin, biglycan, versican, perlecan, dermapontin
  - Matrix proteases and regulators: MMPs (2,7,12,13,23) and TIMPs (1-3), ADAMs, ADAMTSs, ADAMTSLs
  - Profibrotic genes: TGFβ & Rc, IL-2R, IL-34, LOX & LOXL, annexins
  - Involved in HSC activation (fibrinogenesis): Gal-3, IL-33, PDGF, fascin

Representative genes **down-regulated** in liver of DIO-NASH mice and recovered by treatment with SJT4A:

- Involved in regulation of energy expenditure: MUPs
- Involved in inflammation and macrophage recruitment: MARCO
Toxicology studies

• Early toxicology studies indicate an encouraging safety profile
• Acute toxicity in male mice (Irwin test)
  – No deaths or behavioral disorders observed
• Repeat-dose toxicity in male mice
  – Repeat dose up to 250 mg/kg daily for 14 days, by oral administration
  – Neither systemic toxicity nor relevant toxicity in the major functional organs
  – No deaths at the end of treatment
  – No effects on the weight of mice
• Genotoxicity
  – No mutagenic activity detected with the bacterial reverse mutation test (Ames test)
  – No chromosomal aberrations observed with an in vivo micronucleus test in mice at concentrations up to 500 mg/kg, indicating a lack of bone marrow toxicity
  – No genotoxicity in *in vivo* bone marrow micronucleus assay
    • No evidence of clastogenicity or aneugenicity in 9 male and 9 female mice (1-2 oral administration, up to MTD* of 750 mg/kg/day in male and 500 mg/kg/day in female mice)

* MTD: Maximum tolerated dose
Hypothesis of MoA, Dual Action: SJT4A is an agonist for the FXR and an antagonist for the 5-HT2AR

The study was carried out by Softmining, using advanced computer systems and artificial intelligence in supercomputer analyzing the atomic structure of 284 crystallographic structures candidates for interaction with SJT4A.

The 284 candidate crystallographic structures were selected by prior analysis of the atomic structure of SJT4A and other NASH-related molecules. These 284 structures resulted in 30 receptors belonging to 7 different families. Once these potential targets were identified, computational studies were performed on 1) molecular anchorage, 2) molecular dynamics, 3) calculation of binding energy and 4) calculation of residence time.

The FXR receptor obtained a high binding affinity (10.2 kcal/mol; 35 nM) and a long residence time in the activation pocket (21 a.u.). The 5-HT2AR obtained also a high binding affinity (9.99 kcal/mol; 48 nM) and a long residence time in the activation pocket (1.20 ns). These results indicate the specific binding of SJT4A to the FXR and 5-HT2A receptor.

PS: For details please read the MoA documents part I and part II provided
The farnesoid receptor X (FXR) is a nuclear receptor (transcription factor) sensitive to bile acids that regulates lipid, cholesterol and glucose metabolism. FXR is highly expressed in the liver and intestines, and is also found in the kidney and adrenal glands. FXR has a ligand-independent functional transcription activation (AF-1) domain, a DNA binding domain with two highly conserved zinc finger motifs, and a hinge region that mediates simultaneous receptor dimerization and DNA binding at the N-terminal end. At the C-terminal end of the FXR there is the ligand binding domain, a dimerization interface and a ligand-dependent AF-2 domain.

The FXR dimerizes with the retinoid receptor X-alpha (RXRα), another nuclear receptor, which allows the FXR to bind to a DNA sequence called inverted repeat-1 response element (IR-1) to initiate the transcription of the target genes.
The FXR receptor (II)

SJT4A is an agonist for the FXR transcription factor

SJT4A binds to the FXR receptor activating its interaction with the IR1 element promoting the transcription of the FXR-target genes which causes a decrease in triglyceride synthesis and plasma triglyceride levels together with the increase of triglyceride clearance.
In the initial state of interaction, the presence of SJT4A causes the opening of the interaction pocket in the FXR protein. In the left figure the interaction domain (light grey) is closed. The figure on the right shows how the presence of SJT4A opens this interaction pocket.
Opening and closing the activation pocket. The pocket opens in the presence of SJT4A (left) and closes after the penetration of SJT4A into the FXR. The amino acids involved in these interactions are shaded in red. The amino acids and their position are shown in blue.
The FXR receptor (V)

FXR Binding Pocket. The figure shows the union of SJT4A and the main amino acids involved and their positions.
The 5-HT2A receptor (I)

The 5-HT2A receptor is a subtype of the 5-HT2 receptor that belongs to the serotonin receptor family and is a G protein-coupled receptor (GPCR). The 5-HT2A receptor is expressed in the liver and its expression is markedly increased after high fructose diet in animal models. Very recently the 5-HT2A receptor activation has been found to induce hepatic liver steatosis.

Interaction of SJT4A with the 5-HT2A receptor

SJT4A (light blue) enters to the binding pocket of 5-HT2AR inhibiting its function.
The 5-HT2A receptor (II)

SJT4A is an antagonist of 5HT$_{2A}$ Receptor

SJT4A, acting as a 5HT$_{2A}$ Receptor antagonist is able to regulate several physiological functions, including insulin resistance, lipid metabolism, oxidative stress, inflammation and fibrosis, which also contribute to NAFLD and NASH alleviation.
Summary

• Novel family of **oral molecules**
• Innovative mode of action with potential application in:
  – NAFLD (NASH)
  – Type 2 diabetes
  – Obesity
  – Hypertension
  – Dyslipidemia and diabetes associated complications
• **Patent-protected molecules** with long expiry dates in major markets
• Potential **first in class** therapeutics based on β-carboline structure
• **Efficacy demonstrated** in *in vivo* animal models
• Encouraging **safety profile from early toxicology studies**
• “First in Class” novel **dual mechanism of action (FXR agonist and 5-HTR$_2$A antagonist)**
• **Simple manufacturing**: 3-4 step chemical synthesis with high yield and purity (>99 %)
• Partners sought for further development and commercialisation of SJT’s novel, proprietary molecules
• Flexibility in deal structuring: licensing, co-development, investor,..
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