ABTL0812 IN NEUROBLASTOMA

AbilityPharma

Ability Pharmaceuticals, SL, is a biopharmaceutical company developing new **first-in-class drug** candidates to **treat cancer, including neuroblastoma and pediatric cancers**. We are focused on **autophagy as a new therapeutic strategy to induce cancer cell death**. Based on this mechanism of action, we are generating a portfolio of new drugs that will target oncological unmet needs. The company was established in 2009 with a first candidate, ABTL0812, an **oral autophagy-inducer targeted therapy**, currently, at **phase 2 clinical stage**. AbilityPharma's team is formed by 11 highly qualified professionals, each one with long experience in one of the key areas of the biotech and pharma industry: basic oncology research, genetic characterization of cancer, clinical development of oncology drugs, regulatory affairs, IP protection and business development and licensing. The management team has a track record in getting drugs approved by EMA and FDA, transferring technologies and licensing products to big pharmaceutical companies, and managing a growing biotech company. Additionally, we have an extensive collaboration network that includes Key Opinion Leaders (KOLs) from academia, clinics and industry. In the pediatric cancer area, our collaborators include: Dr Miquel Segura (Principal Investigator, VHIR); Dr Soledad Gallego (Head of Pediatric Oncology and Hematology, VHIR); Dr Gilles Vassal (Head of Clinical Research, Institut Gustave Roussy); Dr Birgit Geoerger (Head of the Pediatric New Drug Development program, Institut Gustave Roussy); and Dr Peter Adamson (Chairman of the Children's Oncology Group at Children's Hospital of Philadelphia).

ABTL0812

ABTL0812 is a **first-in-class oral targeted anticancer** compound that produces autophagy-mediated cytotoxicity selectively in cancer cells. ABTL0812 binds and activates the transcriptional activity of the nuclear receptors PPARα and PPARγ, leading to the **induction of Endoplasmic Reticular Stress (ER-stress**), and to the **blockade of Akt activation**, the central kinase of the PI3K/Akt/mTOR pathway (Figure 1). This dual action of ER stress activation and Akt/mTOR axis blockade converge to strongly induce **cytotoxic autophagy**, which results in **cancer cell death**. Very importantly, at therapeutic concentrations, ABTL0812 **does not kill normal cells**, being highly selective for tumor cells[1,2].

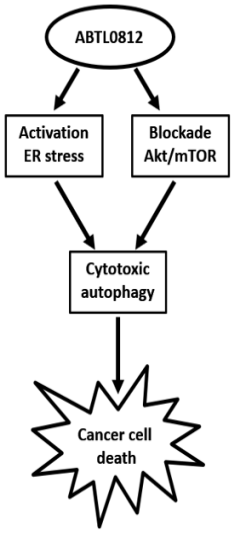

Figure 1. ABTL0812 mechanism of action. A) ABTL0812 has a dual anticancer action: 1) ER stress activation and 2) Akt-mTOR blockade. Both actions converge in the induction of a robust cytotoxic autophagy that leads to cancer cell death.

Preclinical data

The **anticancer activity** of ABTL0812 has been shown in a broad array of tumor cell lines and animal models, including **neuroblastoma, glioblastoma, cholangiocarcinoma, endometrial**[3]**, lung**[4]**, breast, ovarian, prostate and pancreatic cancers**. In addition to the efficacy of ABTL0812 as a single therapy, we have demonstrated that ABTL0812 **potentiates chemotherapy efficacy without increasing toxicity**. This potentiation effect has been shown for a number of chemotherapy drugs in different cancer animal models: carboplatin+paclitaxel in endometrial cancer[3]; docetaxel, paclitaxel, carboplatin+paclitaxel and pemetrexed in lung cancer[4]; gemcitabine, gemcitabine+paclitaxel and gemcitabine+nab-paclitaxel in pancreatic cancer; and temozolomide in glioblastoma. These preclinical data are consistent with the observed potentiation of chemotherapy by ABTL0812 in human patients in our ongoing phase 2 clinical trial (see below). Our preclinical data in **neuroblastoma, pancreatic cancer and cholangiocarcinoma** models led to the obtention of **Orphan Drug Designation** (ODD) for these indications by the FDA and EMA.

Clinical data

ABTL0812 successfully concluded a **First-in-Human Phase 1** clinical trial (EudraCT Number 2013-001293-17) in advance solid tumors, where it was demonstrated its **high safety and tolerability**. The recommended Phase 2 dose (RP2D) was determined based on pharmacokinetic/pharmacodynamic modelling, since no Dose-Limiting Toxicities (DLTs) were detected and a Maximum Tolerated Dose (MTD) was not achieved[5]. Additionally, several **long disease stabilizations** up to 18 months were found, indicating potential signs of efficacy. These promising results allowed the initiation of an ongoing **Phase 1/2 clinical trial**, where ABTL0812 is given as first line therapy in **combination with paclitaxel and carboplatin** (P/C) in patients with advanced **endometrial cancer or squamous non-small cell lung carcinoma (NSCLC)** (EudraCT 2016-001352-21). The same protocol has been approved by the FDA (IND 137394). The trial is currently ongoing in Spain and France. To date, the phase 1 part has been successfully completed, detecting that the combination of **ABTL0812 and P/C chemotherapy is safe and well tolerated**[6]. The phase 2 interim results have shown not only disease stabilizations, but **long-term responses**. In the endometrial cancer arm, ABTL0812 combined with P/C increases by almost 30% the efficacy of P/C alone, i.e. ORR (objective response rate) is increased from 51% to 66%. In the squamous NSCLC arm, ABTL0812 combined with P/C increases progression free survival (PFS) from 4.2 to 6.1 months a 45% increase survival vs. P/C alone.

AbilityPharma has recently received a **European EIC accelerator grant** from the H2020 program to perform a **phase 2 clinical trial** to study ABTL0812 in combination with the chemotherapy combo FOLFIRINOX in advanced metastatic **pancreatic cancer**.

ABTL0812 preclinical data in neuroblastoma and other pediatric cancers

Preclinical data has shown the potential therapeutic effect of ABTL0812 in neuroblastoma and other pediatric cancers. The following has been demonstrated:

1- ABTL0812 inhibits growth of neuroblastoma cells *in vitro* regardless of their drug-resistant phenotype and their genetic alterations.

2- ABTL0812 is not mutagenic and does not induce DNA damage, thereby providing a good high safety profile.

3- ABTL0812 has good tolerability *in vivo*. As a single therapy has a similar therapeutic efficacy to cisplatin and when combined ABTL0812 potentiates the effect of cisplatin.

4- ABTL0812 induces ER stress leading to autophagy and cell death in neuroblastoma cell lines.

5- ABTL0812 decreases MYCN expression, a major driver in neuroblastoma and pediatric cancer tumorigenesis.

6- ABTL0812 enhances the antitumoral effect of the chemotherapeutic drugs such as irinotecan.

7- The combination of ABTL0812 with the differentiating agent 13-cis-retinoic acid have a synergistic effect.

Research plan

Objective #1: Evaluation of ABTL0812 as single therapy and in combination with irinotecan and retinoic acid in subcutaneous xenografts mouse model

Here we will study the combination of ABTL0812 with irinotecan and retinoic acid, drugs that have previously shown enhanced efficacy when combined with ABTL0812. Subcutaneous xenografts will be established injecting $5x10^6$ SK-N-BE(2) cells in the flank of immunodeficient mice. Once the tumors have been established, mice will be randomized and treated with 120 mg/Kg of ABTL0812, 5mg/Kg Irinotecan or 100 mg/Kg Retinoic Acid as single agents and in combination (n=10/group). Tumors will be measured 2 times per week using a digital caliper.

Objective #2: Evaluation of ABTL0812 as single therapy and in combination with irinotecan and retinoic acid in metastatic models of neuroblastoma.

Objective 2.1: Liver metastasis model

We propose to test ABTL0812 in a model more representative of advanced disease, i.e. tumor growth in distant organs frequently found in metastatic neuroblastoma. Growth of neuroblastoma in the liver will be established by the tail vein injection of SK-N-BE(2), that has a major tropism to the liver (Figure 2). Between, 10-$30x10^3$ luciferase-tagged cells will be injected in the lateral vein of immunodeficient mice. Once the metastasis have been established (~15 days), mice will be randomized and treated with 120 mg/Kg of ABTL0812, 5mg/Kg Irinotecan or 100 mg/Kg Retinoic Acid as single agents and in combination (n=10/group). Metastatic growth will be measured once a week using In vivo bioluminescence imaging.

Objective 2.2: Lymph node metastasis model

Growth of neuroblastoma in the lymph nodes will be established by the tail vein injection of the Kelly neuroblastoma cell line that has a specific tropism for the lymph nodes (Figure 2). Between, 1-$3x10^5$ cells will be injected in the lateral vein of immunodeficient mice. Once the metastasis have been established (~15 days), mice will be randomized and treated with 120 mg/Kg of ABTL0812, 5mg/Kg Irinotecan or 100 mg/Kg Retinoic Acid as single agents and in combination (n=10/group). Metastatic growth will be measured once a week using In vivo bioluminescence imaging.

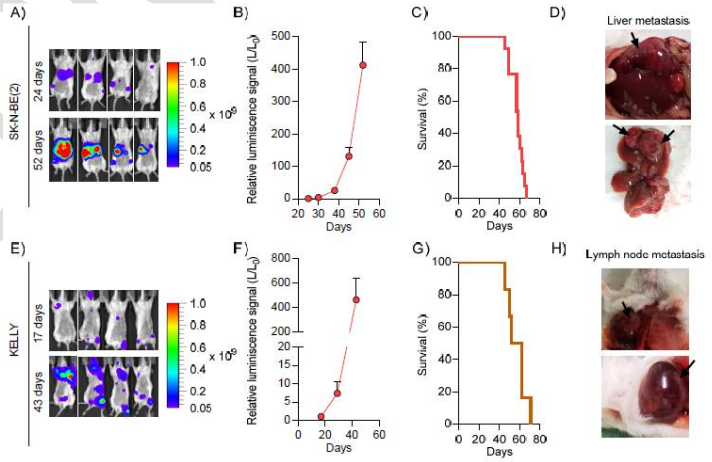

Figure 2| (A,E) A representative image comparing the initial and the final bioluminescence activity (photons/second). (B,F) Relative bioluminescence over time. (C-G) Kaplan-Meier survival curve of mice with metastasis. Results are expressed as mean ± S.E.M. (D-H) Representative images of liver and lymph nodes metastasis originated in mice from SK-N-BE(2) and KELLY cell lines after tail vein injection, respectively.

Objective #3: Evaluation of ABTL0812+ anti-GD2 in immunocompetent mice.

Current multimodal treatments for patients with neuroblastoma (NBL), including anti-disialoganglioside (GD2) monoclonal antibody (mAb) based immunotherapy, result in a favorable outcome in around only half of the patients with advanced disease. We will test the effect of combining ABTL0812 with Dinutuximab in TH-MYCN mouse derived cell lines (e.g. 9464D). Subcutaneous xenografts will be established in immunocompetent mice ($1x10^6$ cells/flank). After tumor engraftment, mice will be randomized and treated with 120 mg/Kg of ABTL0812 (oral), 200µg anti-GD2 (i.p) as single agents and in combination (n=10/group). . Injections of anti-GD2 or isotype control Ab were repeated four times per week. Tumors will be measured 2 times per week using a digital caliper.

Objective #4 (Optional): Patient-Derived Orthotopic Xenografts (PDOX).

Several NB PDOXs have already been generated through implantation of intact patient tissue fragments into the adrenal gland of immunodeficient mice. Our NB PDOXs retain the protein expression profile, gene copy number changes, mutational profile, differentiation status and stromal hallmarks of their corresponding patients' tumors (Table 1). Cells ($1x10^6$ cells in a volume of 30 µl) or small tissue fragments will be injected into the adrenal gland of immunodeficient mice (NMRI Nude mice, Harlan). After tumour engraftment, mice will be randomized (n=5-7/group) and treated with vehicle or ABTL0812 120mg/kg will be administered by ip twice a week. At termination, tumor, spleen, kidney, liver and lungs will be collected for histological analyses (i.e Ki67/active Caspase-3). Only the best combination found in the subcutaneous xenografts will be tested.

Table 1. Neuroblastoma PDOX generated at VHIR (Collaboration Xenopat SL)

PDOX ID	Age at diagnosis (mo)	Sample Source	Molecular Alterations
T523	5	Primary tumor suprarenal gland. Post-induction	MYCN, NAG and DDX1 amplification. ALK wild-type, not amplified NCA: no SGA: -1p, +17q, -2q y -10q
T566	20	Cerebellar metastasis of PDOXT523	MYCN, NAG and DDX1 amplification. ALK wild-type, not amplified NCA: no SGA: -1p, +17q, -2q y -10q
T565	3	Hepatic metastasis. Post induction.	MYCN and ALK wild type.
T608	7	Primary tumor suprarenal gland. Pre-treatment.	MYCN amplified SCA:-1p, +2p, +17q, NCA: no NAG, DDX1, ALK non-amplified. ALK p.G1128A (43.77%)

BUDGET

Concept	**YEAR1**	**YEAR 2**	**Total (€)**
Personnel (Junior postdoctoral researcher)	35,000	35,000	70,000
Drugs (ABTL0812, Irinotecan, Retinoic Acid)	10,000	10,000	20,000
Immunodeficient mice xenografts (n=50):	6,000	3,000	6,000
Immunodeficient mice metastases (n=80):	4,000	4,000	8,000
PDOX		30,000	30,000
Facilities (animal facility, imaging platform)	5,000	5,000	10,000
Cell culture reagents	5,000	5,000	10,000
Total	65,000	65,000	130,000

References

[1]Erazo T et al. The New Antitumor Drug ABTL0812 Inhibits the Akt/mTORC1 Axis by Upregulating Tribbles-3 Pseudokinase. *Clin Cancer Res.* 2016 May 15;22(10):2508-19.

[2]Muñoz et al. The anticancer drug ABTL0812 induces ER stress-mediated cytotoxic autophagy by increasing dihydroceramide levels in cancer cells. *Autophagy*. (Forthcoming)

[3]Felip I. et al. Therapeutic potential of the new TRIB3-mediated cell autophagy anticancer drug ABTL0812 in endometrial cancer. *Gynecol. Oncol*. 153 (2), 425–435 (2019).

[4]López-Plana A at al. The novel proautophagy anticancer drug ABTL0812 potentiates chemotherapy in adenocarcinoma and squamous nonsmall cell lung cancer. *Int J Cancer*. (2020)

[5]Alfon, J. et al. Determination of recommended phase II dose of ABTL0812, a novel regulator of Akt/mTOR axis, by pharmacokinetic-pharmacodynamic modelling. *Ann. Oncol*. 27, (2016).

[6]Fariñas-Madrid, L et al. Phase 1 of ABTL0812, a proautophagic drug, in combination with paclitaxel and carboplatin at first-line in advanced endometrial cancer and squamous cell lung carcinoma. *J. Clin. Oncol*. 37:15_suppl, 3089-3089 (2019)