SUPPORTING INNOVATION AND TECHNOLOGY TRANSFER IN ONCOLOGY

STARK

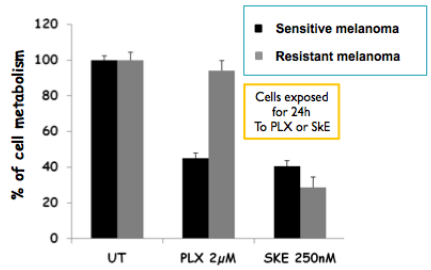
SIMALIKALACTONE E: A NEW NATURAL PRODUCT ACTIVE *IN VIVO* AGAINST DRUG RESISTANT HUMAN TUMORS (*MELANOMA, LEUKEMIA, AND OTHER MAPK DRIVEN CANCERS*)

2013_Deharo

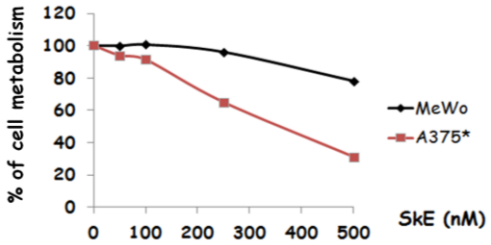
SCOPE	**Optimization of a Lead to Drug candidate nomination**
KEYWORDS	***Simalikalactone, MAPK driven cancers, resistance, B-Raf V600E mutation***

CONTEXT & BACKGROUND

In many resistant cancers, the Ras-Raf-MEK-ERK (MAPK) signalling pathway is constitutively activated through multiple mechanisms. Some specific inhibitors of this pathway have been commercialized but the emergence of B-Raf mutation leads to the resurgence of the tumoral process quickly after the beginning of the treatment.
A 10-years research program on bioactive natural compounds from South American biodiversity lead to the discovery of a new anticancer molecule, the simalikalactone E (SkE), isolated from the leaves of a shrub: *Quassia amara* L. (Simaroubaceae).
SkE affects the MAPK pathway **including B-RAFV600E mutated cells**. SkE abrogates MEK1/2 and B-Raf phosphorylation, but has no effect on Ras activity. SkE is active: ***in vivo*** on CML, *in vitro* on **BRAFV600E mutated** cells (HCL and **melanoma)** and on Karpas 299 B lymphoma cells.



*SkE re-sensitizes the PLX-4032-resistant 451Lu **melanoma** cell line*



*SkE is more active on **Melanoma cells** with BRAFV600E mutation (red)*

INNOVATIVE COMPONENT & TECHNOLOGY

SkE has been tested for two different indications, leukemia and melanoma, and shows a very good activity in both of them:

- in the high nM range SkE is efficient at 1µM, to inhibit colony formation in K562 **CML** cell line, and ***in vivo*** to inhibit tumor formation in a xenograft model of CML cells in athymic mice (1mg/kg, **a dose 60 time lower than Imatinib**).
- SkE induces cell death more efficiently than Crizotinib in Karpas 299 **B lymphoma cell** line.
- SkE is as efficient as PLX (Vemurafenib) in primary B cells from **hairy cell leukaemia** with the BRAFV600E mutation.
- SkE is efficient to induce loss of cell metabolism in **melanoma** cells harbouring the **BRAFV600E mutation and to a lesser extent in a BRAF wild-type melanoma**.
- **SkE re-sensitizes the PLX-4032-resistant 451Lu melanoma cell line**

OBJECTIVES

To pursue development of SkE as a cancer therapy for melanoma, by realising the necessary steps to bring the molecule from a TRL 3 level to a TRL 4-5 level. These steps comprise the scale-up and optimisation of current extraction method; studies of biological activity in combination with reference compounds and well as MoA; and the determination of the safety/activity index in other models than mice; the galenic formulation; and the ADME parameters.

TARGET POPULATION

Treatment of drug resistant melanoma and leukemia (populations bearing BRAFV600E mutation)

TARGET PROFILE

→ **High score in production**: *Quassia* is an easy to obtain plant, cultivated for its wood. SKE is extracted from leaves, considered to be a waste from the wood industrial production. The extraction process has been defined in semi-pilot environment (yield of 0,004%).
→ **High score in resistant cells**: more efficient than U0126, a MEK inhibitor and PLX-4032 to induce apoptosis in Hairy Cell Leukemia (HCL) patient samples carrying the B-Raf V600E mutation. More efficient on mutated melanoma cells than non mutated cells.

→ **Medium score in administration**: SkE inhibits tumor formation in a xenograft model of CML cells in athymic mice, by IP route (at 1mg/kg IP). SkE has been proved to be active in mice by **oral route** against fast replicative protozoa: *P. berghei*.
→ **Medium score in Safety**: no acute toxicity has been showed by IP route in mice for 18 days on alternate daily basis administration; doses up to 50 mg/Kg have been administrated by IV route without any clinical sign of toxicity.
→ **Low score in formulation**: SkE has been administrated in DMA and must be formulated for oral administration
→ **Medium score in PK**: dosage process has been defined by LCMS, follow up of biodisponibility must be set up
→ **Medium score in Stability**: shelf stability has been roughly defined (1 year).

DEVELOPMENT & MATURATION STAGE

- SkE has been tested both *in vitro* and *in vivo* in two different models, but one needs to confirm therapeutic indication.
- We have shown that SkE inhibits the MAPK pathway but the molecular target remains unknown. The precise mode of action of SkE will be addressed by a kinome screening assay.
- Preliminary results suggest SkE has a low level of toxicity: besides the tests in mice, the molecule is a component of an herbal tea used in traditional medicine. Its safety needs to be confirmed by tests in other animal models.
- Extraction of SkE is currently a small-scale laboratory process. A scale-up from 10kg of fresh leaves to several tons is necessary in order to achieve industrial production of the molecule. Fresh leaves provision will not be a problem (2 different providers in South America, with strong experience in Quassia culture have already been identified). We have developed a very reliable SkE quantitation protocol that allows to pilot optimization of the extraction process easily.
- Other pre-clinical studies are yet to be performed: Galenic formulation must be defined for oral and IV route; and PK and ADMET data must be determined.

INTELLECTUAL PROPERTY & PATENT CO-OWNER(S)

2 patent families owned by Institut de Recherche pour le Développement (IRD), and covering Europe, USA, Japan, Canada, Brazil and India:

- WO/2012/095820 – "Use of Simalikalactone E as an anticancer agent", priority 14/01/2011
- WO/2010/146257 – "Simalikalactone E and use thereof as a medicament", priority 18/06/2009 – **Granted in EP & US**

STRENGHTS & COMPETITIVE ADVANTAGES

- Plants have been a source of medicine throughout history and continue to serve as the basis for more than 25% of pharmaceuticals used today. This percentage rises up to 50 % in the case of cancer.
- The molecule is easily extracted from a plant that is cultivated for its wood. The extraction process can be easily monitored. Furthermore, hemi-synthesis can be an alternative to enhance yields of production.
- No competition with other uses of the plant, leaves are considered to be a waste.
- SkE fulfils an unmet need, as a treatment to counteract resistance to current drugs (in a growing number of patients).

INDUSTRIAL APPLICATIONS & PARTNERSHIP OPPORTUNITIES

Hairy Cell Leukemia: there are approximately 1,000 and 1,600 new cases per year of HCL in the US and in Europe respectively, around 10% of them being resistant to therapy. Cost for 2nd line therapy : around 4000 euros for Rituximab and a 24 weeks course of interferon alpha cost around 7000 euros.
Melanoma: **Evolution of the Marketed drug $1.1bn in 2012** to **$2.4bn by 2021** (compound annual growth rate (CAGR) of 9.24%). 50% of the cases are driven by mutation in the BRAF gene; 60-80 % initially responds to BRAF inhibitors but most develop resistance within 7-8 months. Main completion in this market is:

→ *Vaccines*: used in patients with stage IV melanomas but with very low response rates. The interleukine 2 (Proleukine, Novartis) stimulates T-lymphocytes growth but tolerance in patients is very low (the injection is particularly painful).

→ *Immune checkpoint inhibitors*: Ipilimumab (MDX-010, marketed as Yervoy by Bristol-Myers Squibb), a human monoclonal antibody, has been utilized to inhibit CTLA-4 and thus promote anti-tumor immunity. It is used to induce tumor regression and block immunosuppression in advanced melanoma patients but the median survival rate does not exceed 10 months.

→ *Chemotherapy*: Several treatments exist but are far from being perfect. (1) Dacarbazine shows poor response, increasing the median survival in only 7.5% of treated patients for no more than 18 months. Administrated by IV route, it is considered to be highly emetogenic and toxic for liver. (2) Temozolomide is an oral alkylating agent and a dacarbazin related drug used also for the treatment of melanoma. (3) In a Phase III, randomized placebo-controlled study with unresectable stage III or IV melanoma patients, Sorafenib, did not improve any of the end points measured over placebo plus carboplatin–paclitaxel. (4) Vemurafenib (INN, marketed as Zelboraf) is a B-Raf enzyme inhibitor but the regression only lasts from 2 to 18 months.

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