REPOSITIONING ER005 FOR THE TREATMENT OF RARE AND PEDIATRIC CANCERS

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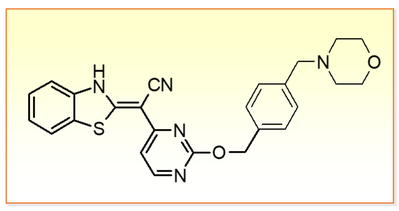
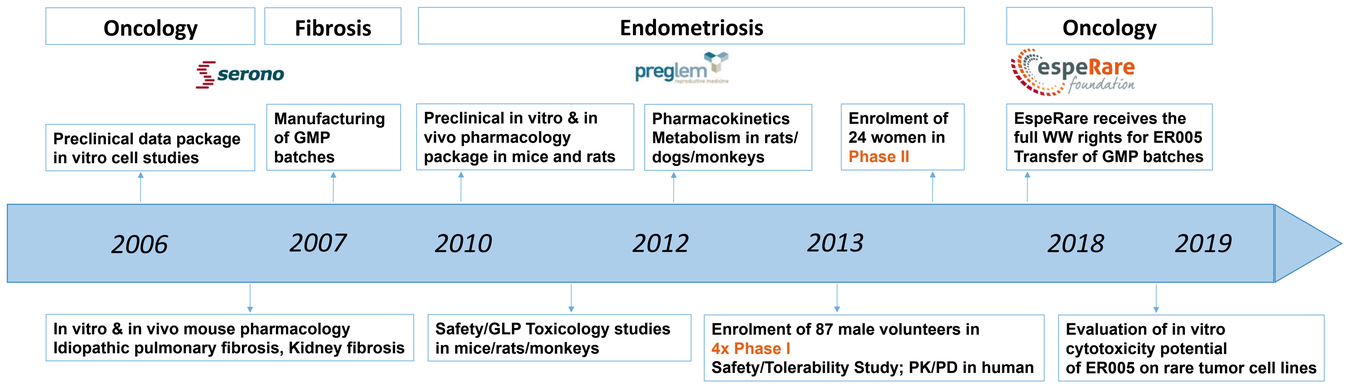
Introduction

Background of ER005



ER005 (Bentamapimod, AS602801) c-jun N-terminal kinase (JNK) inhibitor

- JNKs are MAP kinases that regulate inflammation, morphogenesis, cell proliferation, differentiation, survival and death
- JNKs are upregulated in many cancers and are associated with drug resistance
- **ER005 is a highly selective first-in-class JNK inhibitor** with IC_{50} = hJNK1 90 nM, hJNK2 230nM,hJNK3 50 nM
- **ER005 inhibits tumor initiating capacity of cancer stem-cells** in mice (first in vivo study with ER005 in cancer model, *Okada 2016*[1])



GLP Toxicology studies and a complete safety PK&PD in healthy adults and endometriosis patients

Extensive Toxicology studies showed that ER005 is safe

- Repeated oral treatment up to 13-weeks in rats and up to 39-weeks in monkeys did not induce any signs of toxicity
- ER005 in single doses of up to 570 mg and ER005 given for 14 days using a twice daily dosing in the range of 5 to 160 mg BID have been well tolerated in 4 studies conducted in healthy volunteers.
- Well tolerated at 160 mg BID for 5 months in a Phase 2 study in women with inflammatory endometriosis
- No effect on cardiovascular hemodynamic parameters, no evidence of risk for any pro-arrhythmic effects
- No relevant effects on the central and peripheral nervous system, nor on the gastro-intestinal, renal and respiratory system

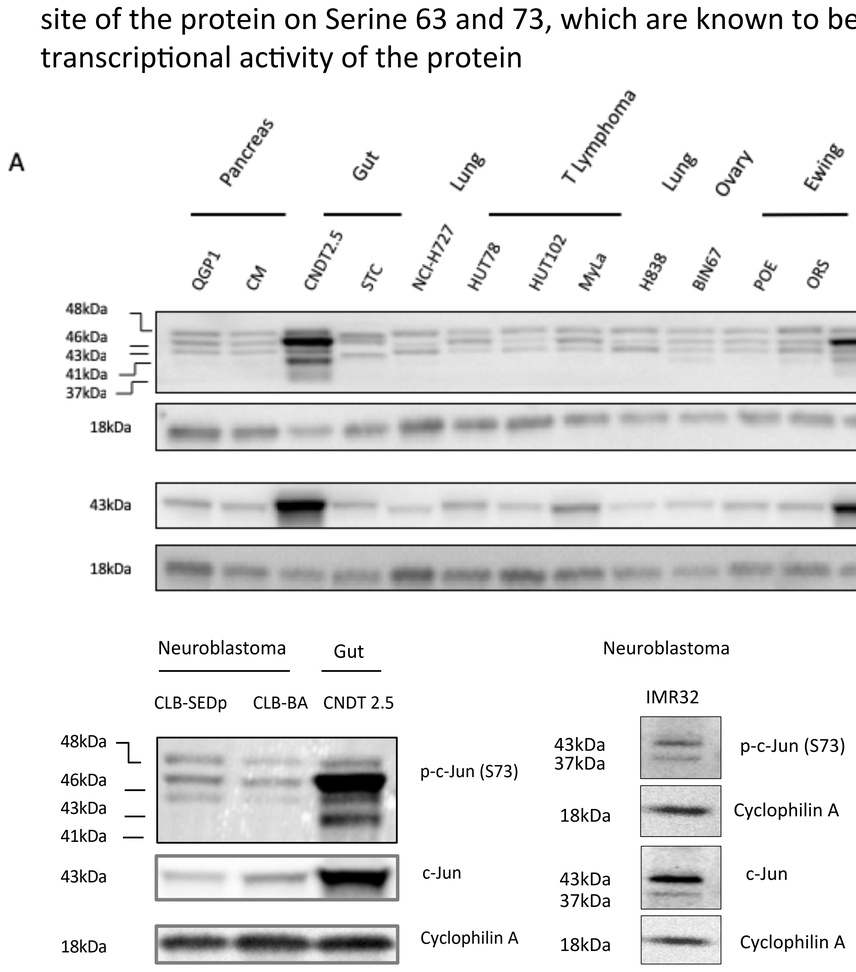
Methods

- **Cytotoxicity in rare cancer cell lines and healthy fibroblasts**: the cytotoxicity of ER005 on a panel of 20 human cancer cell lines (neuroendocrine tumor (NET), neuroblastoma, sarcoma, T lymphoma) and non-cancer cells was determined using the MTT assay
- **JNK activation** was analyzed by quantifying the phosphorylation of c-Jun using Western Blot analysis

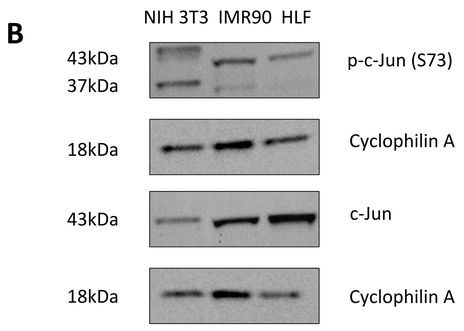
Results : ER005 Repositioning in Oncology – Pediatric and Rare Cancers

The JNK pathway is activated in rare cancer cell lines

- JNK1 and JNK2 are the only known kinases capable to phosphorylate c-Jun on Ser-73 (detected at 48kDa with anti-p−c-jun Ab #3270, Cell Signaling Technology)
- C-jun possesses several phosphorylation sites, two of them being located at the N-terminal site of the protein on Serine 63 and 73, which are known to be involved in an increased transcriptional activity of the protein



In non-cancer cells the JNK pathway is not activated



- In HLF and IMR90 fibroblasts p-c-Jun (48kDa) is not observed, however p-JunD is weakly detected (37kDa) with the anti-p-c-jun antibody
- This suggests that the JNK pathway is only weakly or not at all activated in normal HFL and IMR90 fibroblasts, but might be activated in mouse NIH3T3 fibroblasts.

Fig. 1. Immunoblot analysis of c-Jun and phospho-c-Jun in (A) rare cancer cell lines and (B) normal fibroblasts. Cyclophilin A is used as loading control.

ER005 is a selective inhibitor

- ER005 selectively inhibits cell viability of tumor cells compared to non-tumor cells; the cytotoxicity of ER005 in cancer cells is at least 5-fold higher compared to normal fibroblasts

Tumor cells		IC_{50} (µM)	I_{max}
Ewing sarcoma	ORS	11.7	90
Ewing sarcoma	Poe	10.5	100
Lung	H838	3.4	70
Ovary	BIN67	15.9	100
Rhabdoid	KD	11.0	100
Rhabdoid	MON	5.1	80
Rhabdoidmyosarcoma	IcPCD19	16.1	60
T lymphoma	HUT102	8.9	100
T lymphoma	HUT78	19.3	60
T lymphoma	MyLa	43	50
NET intestine	STC-1	10.4	90
NET intestine	CNDT2.5	10.9	80
NET lung	NCI-H727	10.4	90
NET thyroid	CM	7.4	100
NET pancreatic	QGP1	20.1	70
Neuroblastoma	CLB-SEDp	> 50	na
Neuroblastoma	IMR32	32.5	na
Neuroblastoma	CLB-BA	na	na
Non-tumor cells			
Human lung fibroblasts	HLF	0% inhibition at 50µM	
Human foetal lung	IMR90	0% inhibition at 50µM	
Mouse embryo fibroblast	NIHT3T	≥50	

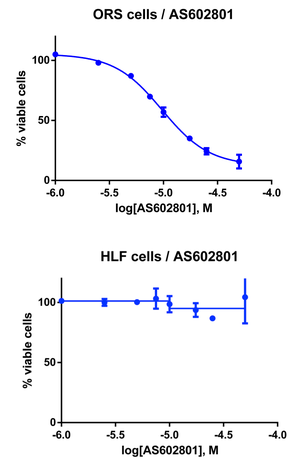
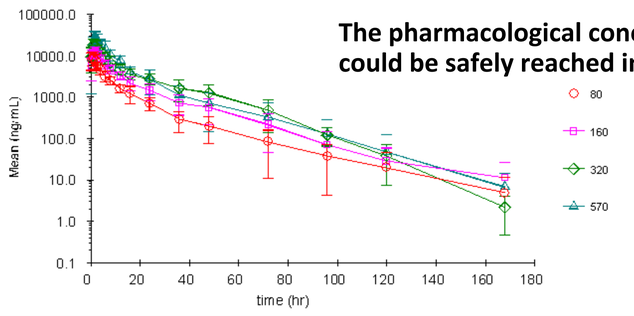


Fig. 2. IC50 and dose response curves of ER005 on several tumor and non-tumor cells determined by the MTT cell viability assay.

ER005 inhibits growth of rare cancer cells in which the JNK pathway is activated

- The cell viability is inhibited in most tumor cell lines with an IC_{50} of 5-15 µM
- ER005 displays some activity in NIH3T3 fibroblasts in which the JNK pathway is activated (phosphorylated JunD seen by Western Blot)
- The IC_{50} could not be correlated with the level of c-jun phosphorylation
- **The association between IC_{50} and the detection of Ser-73 p-c-Jun or p-JunD supports the hypothesis that the cytotoxic activity of ER005 might be linked to targeting the JNK pathway**

PK profile of ER005 in adults



The pharmacological concentrations in the low micromolar range could be safely reached in humans!

Fig. 3. Mean (SD) ER005 Plasma Concentration vs Time Profiles after Oral Administration of 80, 160, 320 and 570 mg (semilog scale). Results from Phase I Clinical Study 26400.

Conclusions

- ER005 is a first-in-class JNK inhibitor that was shown to be safe in humans
- ER005 displayed encouraging cytotoxicity against rare and pediatric cancer cell types at low micromolar concentrations
- ER005 is an ideal candidate to be tested in combination with other chemotherapeutic drugs

References

[1] M. Okada *et al.*, The novel JNK inhibitor AS602801 inhibits cancer stem cells in vitro and in vivo. *Oncotarget*, **7**, 27021–27032 (2016).

Acknowledgements