MPL-7097, an ESM™ p38 MAPK inhibitor

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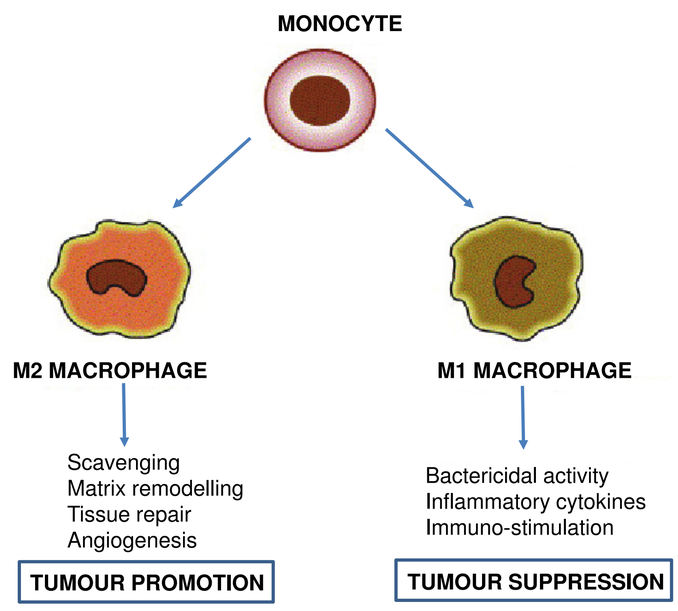
Introduction

• Tumour-associated macrophages (TAMs) contribute significantly to enhanced malignancy in multiple cancers by generating an immunosuppressive tumour microenvironment through production of cytokines such as IL-10.[1,2]

• Polarisation of these immunosuppressive M2 macrophages towards a pro-inflammatory M1 phenotype is capable of activating an effective anti-tumour immune response.[3]

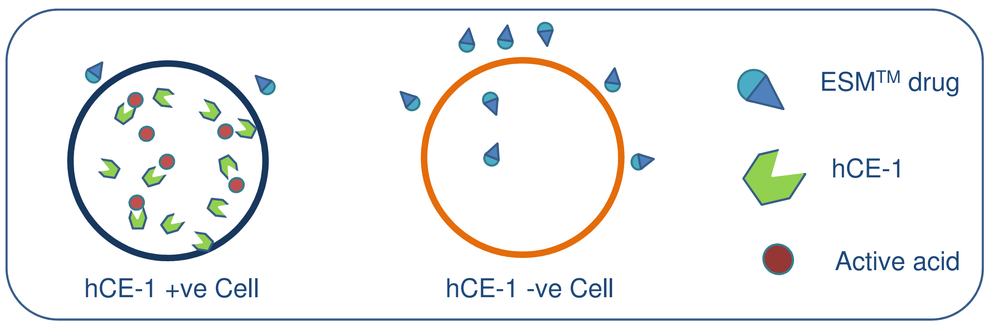


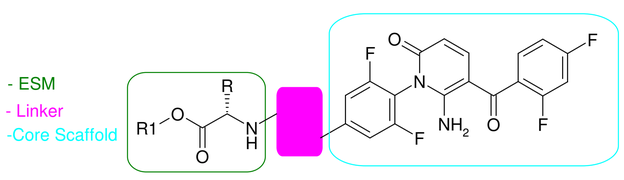
• p38 MAPK has been shown to play a role in polarising macrophages towards an immunosuppressive M2 phenotype, however, it also has a pro-inflammatory effect in other immune cells such as T-cells.[4]

• Macrophage Pharma's Esterase Motif Technology™ (ESM™) targets myelomonocytic cells whilst sparing other immune cells.[5] The application of this technology to p38 MAPK to generate a series of potent ESM™ p38 inhibitors that selectively target myelomonocytic cells will be described.

Esterase Sensitive Motif™ Technology

- Human carboxylesterase, hCE-1 expression is largely restricted to cells of the monocyte lineage; monocytes, macrophages and dendritic cells
- Other human carboxylesterases, hCE-2/3 are ubiquitously expressed
- The ESM™ technology uses esters that are selectively hydrolysed intracellularly by hCE-1
- Accumulation of the pharmacologically active acid occurs in hCE-1 positive cells, since the acid can not readily diffuse out of the cell

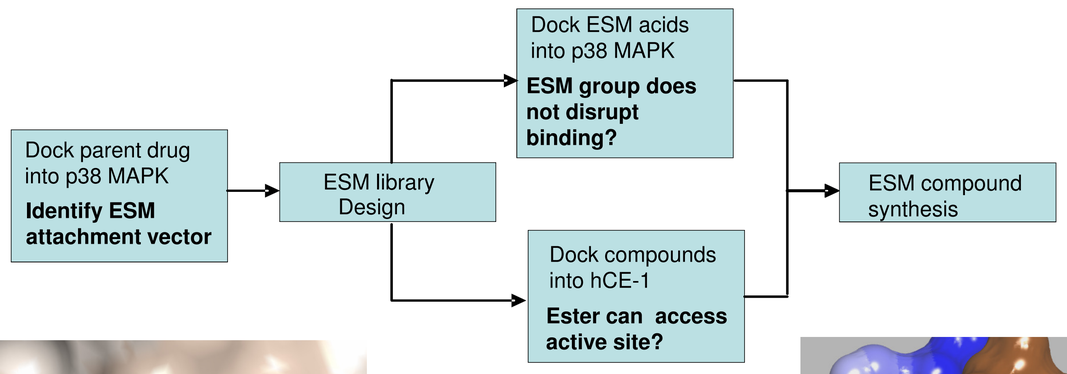


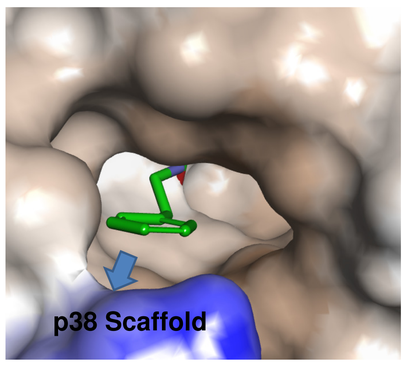


- Macrophage Pharma have developed a p38 MAPK ESM™ inhibitor, **MPL-5821**
- The key structural elements of **MPL-5821** are shown in the diagram (left)

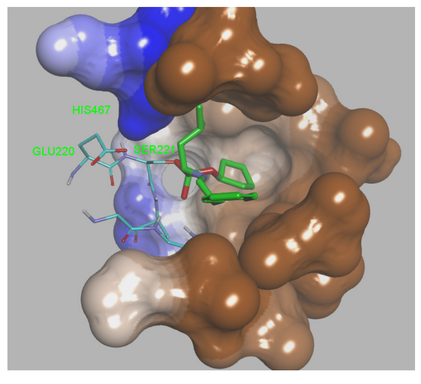
Design of ESM™ p38 MAPK Inhibitors

- With the successful identification of **MPL-5821** as a lead molecule, Macrophage Pharma wishes to investigate the application of their ESM™ technology to other p38 MAPK inhibitor classes
- Alternative scaffolds were investigated and ESM™ compounds designed

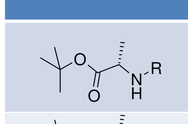
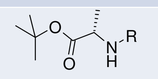
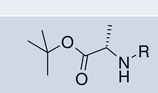
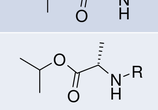
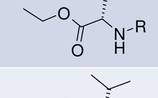
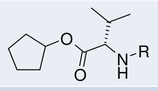
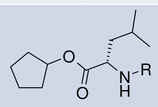




- The ESM™ ester can access the active site of hCE-1 by a narrow cleft (left)
- Typical binding mode of ESM™ compounds to hCE-1 (right)



The rate of hydrolysis by hCE-1 can be modulated by varying the ESM™

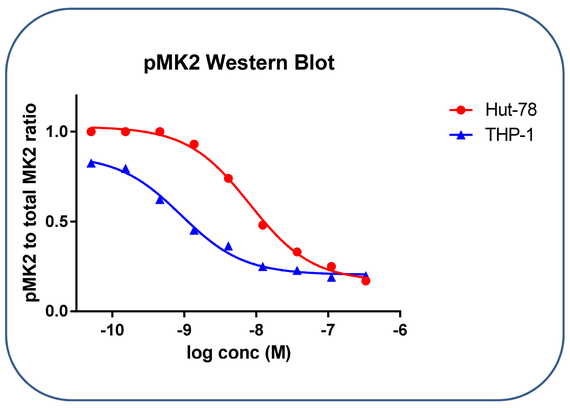
Esterase sensitive Motif™ (R=linker and scaffold)	Linker Type	THP-1 Cell Accumulation Assay Concentration@ 3h (µM)	hCE-1 $t_{1/2}$ (mins)
	2C para	34	892
	2C meta	17	1867
	1C Para	8	2367
	2C para	>571	
	2C para	120	194
	2C para	<5	3958
	2C para	278	21
	2C para	7	

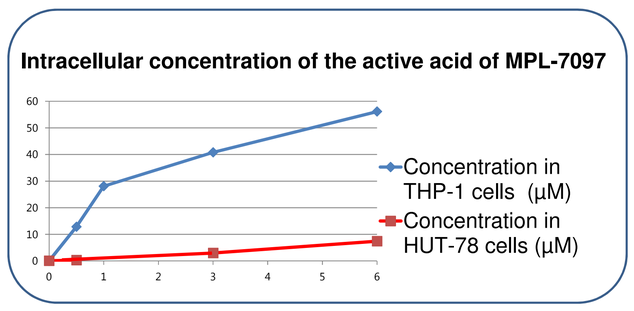
- hCE-1 is also expressed in the liver: therefore, an intermediate rate of hydrolysis was required so that enough ester could avoid first pass metabolism
- A variety of different esterase sensitive motifs™ were assessed to determine the effect the changes had on hydrolysis rate and cell accumulation (select examples shown in the table)
- THP-1 cells (hCE-1 +ve) were used for the cell accumulation assay and the intracellular concentration was determined by mass spectroscopy

MPL-7097

Assay	MPL-7097
p38α MAPK IC_{50} (nM)	2
THP-1 TNFα IC_{50} (nM)	10
Whole blood TNFα IC_{50} (nM)	4
Western blot THP-1 (nM)	9
Western blot HUT-78 (nM)	107

- MPL-7097 was identified as a potent p38 MAPK inhibitor with good cell and whole blood potency.
- MPL-7097 shows selectivity for hCE-1 +ve cells (THP-1) over hCE-1 –ve cells (HUT-78)
- The acid generated from MPL-7097 accumulates to a greater extent in hCE-1 positive cells than in hCE-1 negative cells





ADME/PhysChem	MPL-7097
MW	547
$logD_{7.4}$	3.7
Solubility (pH 7.4 PBS 24 h, µM)	0.3
Human S9 Clint (µL/min/mg protein)	37
CYP3A4 inhibition (µM)	>10
hERG inhibition (µM)	> 10

- MPL-7097 shows good S9 stability.
- MPL-7097 has an excellent ADME profile with no inhibition > 50% at 10 micromolar in CYP3A4 and hERG assays.
- MPL-7097 is selective over other kinases (only 12/356 were inhibited by >50% at 10 µM, data not shown)

Summary

- MPL-7097 was identified as a potent p38 MAPK ESM™ inhibitor
- Macrophage selective drug delivery through application of the ESM™ technology differentiates molecules such as MPL-7097 and MPL-5821 from conventional non-targeted p38 MAPK inhibitors

Services/Contact

Domainex is a fully integrated drug discovery CRO based in the UK. If you would like to learn more about applying our drug-discovery platform to your targets, please contact: **tom.mander@domainex.co.uk**
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References:
1) Biswas *et al*; Semin.Immunopathol; **2013**, 35, 585-600
2) Brown *et al*; Tissue Antigens, **2004**, 64, 3, 215
3) Verreck *et al*; **2004**, PNAS, 101, 13, 4560-4565
4) Yang *et al*; **2016**, Scientific Reports, 1-12
5) Needham *et al*; J.Pharmacol.Exp.Ther., **2011**, 339, 132-142