Characterisation of EXS73565, a Potent and Selective MALT1 Inhibitor with Low Drug-drug Interaction Risk and Potential in Lymphoma

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INTRODUCTION

Mucosa-associated lymphoid tissue lymphoma translocation 1 (MALT1) is a key component of dysregulated antigen signalling pathways in B-cell malignancies.[1] MALT1 protease activity is crucial for activation of the NF-κB pathway which is activated in diffuse large B-cell lymphoma (DLBCL) subtypes.[1] Inhibition of MALT1 may have benefit for haematological malignancies where MALT1 is constitutively activated, such as activated B-cell (ABC)-DLBCL, as a single agent or in combination with BCR signalling pathway modulators such as BTK inhibitors (BTKi).[1,2]

Current MALT1 inhibitors (MALT1i) also inhibit UDP-glucuronosyltransferase 1A1 (UGT1A1), potentially posing a hyperbilirubinaemia risk, which was the most frequently reported adverse event for JNJ-67856633 (JNJ-633) in Phase 1 studies.[3]

Here, we present EXS73565 ('565), a differentiated and highly efficacious MALT1i with minimal UGT1A1 activity, mitigating potential risk of hyperbilirubinaemia.

METHODS

'565 was characterised in a range of preclinical models and ADMET studies:

- Anti-proliferative activity on OCI-Ly3 or TMD8 cells was determined using CellTiter-Glo® after 4 days of treatment
- Proliferation of CLL patient peripheral blood cells cultured for 5 days in the presence of a feeder cell line (S5 cells), CpG, IL-2 and '565 (or DMSO) was determined by flow cytometry using CellTrace™ Violet dye
- *In vivo* studies: Xenografts were grown subcutaneously in NXG mice (OCI-Ly3 model), CB.17 SCID mice (OCI-Ly10 model) and NOD SCID mice (TMD8 model)
- *In vitro* UGT1A1 and transporter inhibition studies performed:

Transporter	Source	Probe Substrate
UGT1A1	Recombinant Supersomes™	Oestradiol
OATP1B1	Overexpressed in HEK293 cells	[^{3}H]-oestradiol 17β-D-glucuronide
OATP1B3		
MRP2	Overexpressed in membrane vesicles (HEK cell-derived)	
BSEP		[^{3}H]-taurocholic acid

RESULTS

Table 1: **'565 is differentiated against competitor compounds**

Parameter	Phase 2 (Janssen JNJ-633)	Phase 1 (Mid-size pharma patent examples)	Phase 1 (Biotech patent examples)	EXS73565
Biochemical IC_{50} (<100 nM)	Minor deviation	Meets or exceeds criteria	Meets or exceeds criteria	Meets or exceeds criteria
OCI-Ly3 IL-10 IC_{50} (<100 nM)	Meets or exceeds criteria	Not tested	Meets or exceeds criteria	Meets or exceeds criteria
OCI-Ly3 proliferation IC_{50} (<400 nM)	Minor deviation	Meets or exceeds criteria	Minor deviation	Meets or exceeds criteria
TMD8 IL-10 IC_{50} (<200 nM)	Minor deviation	Meets or exceeds criteria	Minor deviation	Meets or exceeds criteria
TMD8 proliferation IC_{50} (<300 nM)	Minor deviation	Not tested	Not tested	Meets or exceeds criteria
UGT1A1 IC_{50} (>10 μM)	Major deviation	Major deviation	Major deviation	Meets or exceeds criteria
Hu heps Clint,u (<20 ml/min/kg)	Meets or exceeds criteria	Meets or exceeds criteria	Meets or exceeds criteria	Meets or exceeds criteria
Caco-2 A-B Papp (>5 × 10^{-6} cm/s)	Meets or exceeds criteria	Major deviation	Minor deviation	Meets or exceeds criteria
FaSSIF solubility (>50 μg/mL)	Minor deviation	Not tested	Not tested	Minor deviation
Cerep/full kinase panel	Meets or exceeds criteria	Not tested	Not tested	Meets or exceeds criteria

- Generative AI design employed to deliver '565
- Potent and highly selective
- Allosteric MALT1i
- Favourable ADME properties
- Poor inhibitor of UGT1A1 compared with competitor compounds

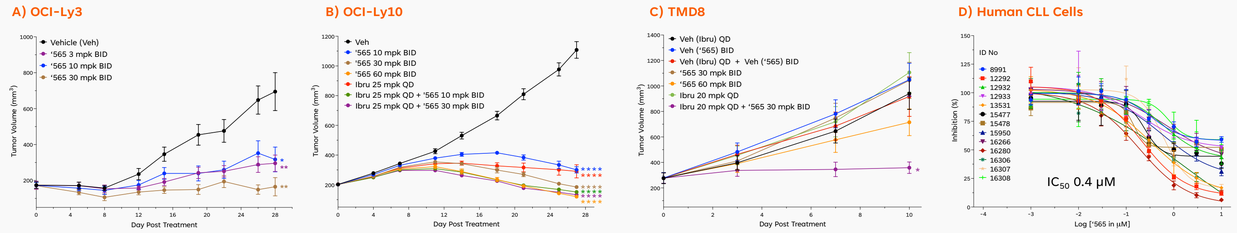
Table 2: **'565 has a low predicted risk of hyperbilirubinaemia**

Compound	Best-estimate Scenario	$C_{max,u}$ ($I_{max,u,inlet}$)	UGT1A1 IC_{50} (μM)	UGT1A1 $IC_{50}/I_{max,u,inlet}$	UGT1A1 R_{free}	UGT1A1 $R_{in,free}$	UGT1A1 F_i	Prediction
JNJ-633	$t_{½}$: 127 h (230 mg QD)	0.28 (0.32) μM	0.76	2.4	1.37	1.42	0.27	Hyperbilirubinaemia Risk
'565	$t_{½}$: 39 h	0.30 (0.42) μM	>10	34	1.02	1.03	0.02	Low Risk

$C_{max,u}$ & $I_{max,u,inlet}$: Predicted maximum free plasma & free liver inlet concentration, respectively. $I_{max,u,inlet} = f_{u,p} \times (C_{max} + (F_a \times F_g \times k_a \times Dose)/Q_h/R_B)$. Predictors ($R_{free}$, $R_{in,free}$, and F_i) used to link clinical hyperbilirubinaemia with enzyme/transporter inhibition. $R_{free} = 1 + C_{max,u}/IC_{50}$; $R_{in,free} = 1 + I_{inlet,max,u}/IC_{50}$; $F_i = 1 - [IC_{50}/(IC_{50} + C_{max,u})]$; Suggested cutoffs: $R_{free} > 1.1$; $R_{in,free} \geq 1.5$; $F_i > 0.2$.[5]

- At predicted human efficacious doses, UGT1A1 $IC_{50}/I_{max,u,inlet}$ margin is 14-fold greater for '565 compared with JNJ-633 (230 mg QD estimate for JNJ-633 comparable with 300 mg QD recommended Phase 2 dose)
- IC_{50}, predictors R_{free} and F_i (based on UGT1A1 inhibition) flag JNJ-633 for hyperbilirubinaemia risk, consistent with Phase 1 clinical findings[3]
- Calculated predictors are below the cutoffs for '565 suggesting minimal hyperbilirubinaemia risk (supported by transporters OATP1B1/3, BSEP and MRP2 inhibition; data not shown)

Figure 1: **'565 is efficacious in ABC-DLBCL xenograft models and primary human CLL cells**



E) ABC-DLBCL Cell Lines Evaluated

Cell line	CD97A/B	CARD11	MYD88	Ibrutinib-sensitive *in vitro*	MALT1i-sensitive *in vitro*
OCI-Ly3	Wild-type (WT)	L244P	L265P	No	Yes
OCI-Ly10	Δ191-208 (CD79A)	WT	L265P	Yes	Yes
TMD8	Y196H (CD79B)	WT	L265P	Yes	Yes

Figure 1: Significant tumour growth inhibition observed for single agent '565 and/or in combination with ibrutinib (Ibru) in **(A)** OCI-Ly3, **(B)** OCI-Ly10, **(C)** TMD8 xenograft models and **(D)** primary human CLL cells. **(E)** Characteristics of ABC-DLBCL cell lines evaluated[2,4]. Tumour volume and % inhibition shown as mean (+/- SEM). P-values determined by mixed model with either repeated measures performed on the log transformed data followed by Tukey's multiple comparison test at each time point (TMD8), or repeated measures performed on day factor followed by Dunnett's comparison test (OCI-Ly3), or two-way ANOVA with Dunnett's comparison test (OCI-Ly10; *P ≤0.05, **P≤0.01, ****P≤0.0001).

DISCUSSION

The selective allosteric MALT1i '565 exhibits *in vitro* anti-proliferative activity (on ABC-DLBCL cell lines and primary human CLL cells) and *in vivo* efficacy in mouse xenograft models both as a single agent or in combination with ibrutinib, including synergistic efficacy in the challenging TMD8 model.

In contrast to competitor MALT1 compounds in development, '565 poorly inhibits UGT1A1, an enzyme involved in bilirubin disposition. We hypothesise that the high UGT1A1 (and transporter) $IC_{50}/I_{max,u,inlet}$ margins at the predicted human efficacious dose for '565 will mitigate potential risks of DDI/hyperbilirubinaemia that could limit dose escalation and the level of target engagement necessary to achieve clinical efficacy.

CONCLUSIONS

- '565 displays favourable properties and offers competitive differentiation, particularly in combination strategies with BTKi
- '565 has potential in a broad range of haematologic malignancies either as a monotherapy or combination therapy
- IND-enabling activities and CMC readiness work are ongoing

Our precision-designed MALT1i '565 has minimal UGT1A1 inhibition risk while maintaining robust potency and selectivity.

REFERENCES

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