Idronoxil levels of patients receiving NOX66

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BACKGROUND

The experimental anti-cancer drug Idronoxil is a first-in-class inhibitor of the oncogene external NADH oxidase Type 2 (ENOX2). ENOX2 maintains the trans-membrane electron potential (TMEP) of the cancer cell plasma membrane, with loss of TMEP disrupting a wide range of functions of the plasma membrane. Inhibition of sphingosine kinase is a major outcome, resulting in loss of a range of pro-survival signalling pathways, notably PI3K and Akt, and consequent loss of function of DNA repair enzymes including PARP 1 and topoisomerases 1 and 2.

Idronoxil was previously investigated as a chemo-sensitiser, utilising its ability to block repair of DNA damage in order to optimise chemotherapy-induced tumour damage whilst minimising non-tumour toxicity. In vitro and in vivo (mouse xenografts) data have shown that idronoxil sensitises by up to 2000-fold the cytotoxic effects of standard cytotoxic drugs including cisplatin, carboplatin, paclitaxel, gemcitabine, toptecan, doxorubicin and captothecin.

Promising early phase results led to a Phase 3 study of oral idronoxil as a sensitiser of carboplatin in patients with carboplatin-refractory ovarian cancer, however this study was discontinued early with data showing no improved efficacy with idronoxil. Subsequent review of the mechanism of action of idronoxil suggested that, for significant biological effect, a constant presence of the parent drug must be present. With a short elimination half life and extensive Phase 2 metabolism in oral and IV formulations, administration of idronoxil in standard form is unsuitable as a drug candidate.

NOX66 is under development as a formulation of idronoxil, specifically designed to overcome the issues identified with oral and IV formulation. Pre-clinical investigations in rats (Table 1) show that NOX66 delivered rectally leads to an extended elimination half life of parent drug in comparison with IV idronoxil.

A first-in-human study of NOX66 as monotherapy and in combination with carboplatin was commenced in March 2017, with preliminary results presented at the ESMO Annual Scientific Meeting in September (summarized below). As part of this study, plasma and urine samples are collected to review and assess the detectable levels of parent idronoxil in patient plasma and urine. Here we outline the assay method, and preliminary finding for monotherapy samples.

Table 1: Comparison of pharmacokinetic parameters of idronoxil administered intravenously in a lipid-free co-solvent formulation and rectally administered NOX66 in rats (n=4 per arm).

Parameter:	IV idronoxil	NOX66 (rectal)
Dose (mg/kg)	3.5[a]	35
Cmax (ng/mL)	4647 ± 315	62.3 ± 8.7
Tmax (h)	-	0.12 ± 0.04
AUC_{0-30} (ng.h/mL)	8129 ± 166	1187 ± 389
Half-life (h)	0.32 ± 0.07[b]	9.6; 6.1[c]
Bioavailability (%F from 0-30h)	100	14.6 ± 4.8

[a] 3.5 mg/kg administered, however PK parameters have been normalised to equivalent of 35 mg/kg

[b] Half life estimate using values from 0.17h. No measurable drug was seen in any rat beyond 2h

[c] Values for 2 rats only

ANALYTICAL METHOD

Plasma and 24h urine samples from 8 patients receiving NOX66 (400mg) were collected and extracts prepared using protein precipitation with acetonitrile then analysed at HMSTrust Laboratory, Monash University. LC-MS analysis was performed on a Shimadzu 8050 triple quadrupole instrument coupled with a Shimadzu Nexera X2 UHPLC. An internal standard of diazepam was used in all samples.

RESULTS

Figures 1-4 show representative chromatograms of control human plasma and standard solvent and of representative plasma and urine extracts from cohort 1 patients.

Chromatograms show internal standard diazepam peak, parent idronoxil peak and metabolite peaks in patient extracts only.

All urine samples analysed have shown detectable levels of parent drug (idronoxil) and metabolites, whilst parent drug was consistently detected in six of eight patients' plasma samples, with all showing evidence of metabolites. Table 2 shows Idronoxil levels measured in plasma (Day 1, 2 hours post NOX66 administration and Day 8, prior to next NOX66 administration) and urine (24h sample from Day 1)

Figure 1: Representative Chromatogram of a Human Plasma Standard (500 ng/mL)

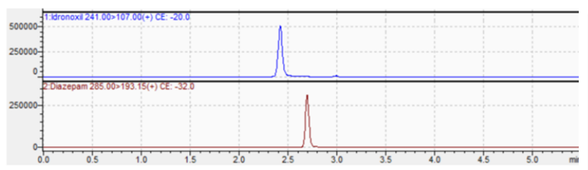

Figure 3: Representative Chromatogram of a Solvent Standard (500 ng/mL)

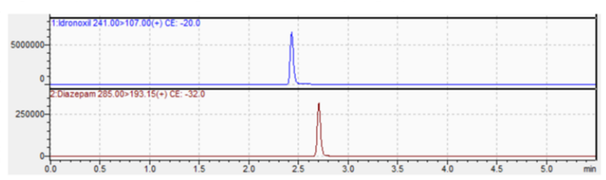

Figure 2: Representative Chromatogram of a Human Plasma Sample (Subject ID 0102, Day 8, 1 hour)

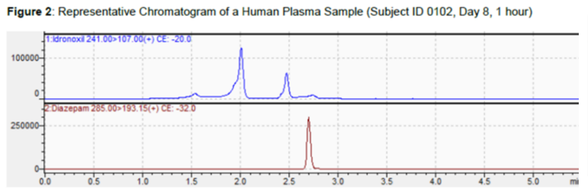

Figure 4: Representative Chromatogram of a Human Urine Sample (Subject ID 0301, Day 1, 24 hours)

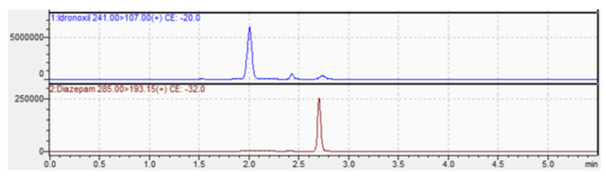

Table 2: Parent idronoxil levels of patients receiving NOX66 400mg.

Plasma samples were collected at 2 hours following administration of first dose of NOX66, and prior to next dose administration on Day 8. post 7 days of consecutive daily NOX66 dosing) 24h Urine sample was collected across Day 1 of administration of NOX66 .

Subject Number	101	102	201	202	203	301	401	402
Patient No.	1	2	3	4	8	5	6	7
Plasma Idronoxil: Day 1, 2h (ng/mL)	6.7	25.2	ND	22.1	15.0	ND	<LLOQ	24.9
Plasma Idronoxil: Day 8, 0h (ng/mL)	5.7	26.4	ND	24.2	9.5	ND	ND	26.9
Urine Idronoxil[a]: Day 1 24h (µg/mL)	0.59	0.22	<LLOQ	0.03	0.05	0.95	0.24	0.20

LLOQ = Lowest Level of Quantification; ND = Not Detected

CONCLUSION

Parent idronoxil can be detected using LC-MS analysis in patients receiving 400mg NOX66 daily, with initial data suggesting a constant presence of parent drug and minimal accumulation. Further PK studies are required to confirm initial findings. Development and confirmation of this method allows for further determination of idronoxil and its metabolites in relation to response to treatment with NOX66.

NOX66-001 Study Overview and Interim Findings

(Presented at ESMO, September 2017)

NOX66-001 STUDY - INTERIM DATA FOR COHORT 1

Pt	Tumour Type	Monotherapy (21 day cycle) Phase 1a Status	Combination Therapy (28 day cycles) Phase 1b Status	Response[#](Cycle 3) Target Lesion RECIST 1.1 criteria	Adverse Events (Phase 1B) ALL	Severity*	Related to NOX66
1	Ovarian	Complete	Ongoing - Cycle 6	Stable Disease Stable disease (Cycle 6)	Nausea	Grade 1/mild	UR
2	Lung	Complete	Withdrawn	Progressive Disease	NIL		
3	Lung	Complete	Ongoing - Cycle 4	Stable Disease	Pulmonary embolism Arterial embolism	Grade 1/mild Grade 1/mild	UR
4	Lung	Complete	Withdrawn (pt decision)	ND			
5	Breast	Complete	Ongoing - Cycle 4	Stable Disease	Exudative pericarditis Bilateral hydrothorax WBC elevation	Grade 1/mild Grade 2/mod Grade 2/mod	UR
6	Breast	Complete	Ongoing - Cycle 3	ND	Hypocalcaemia	Grade 2/mod	UR
7	Breast	Complete	Ongoing - Cycle 3	ND	Asthenia Peripheral neuropathy	Grade 2/mod Grade 1/mild	UR
8	Prostate	Complete	Ongoing - Cycle 3	ND	NIL		

[#] *First response assessment by Investigator; * NCTCAE v4.03 ; ND=Not Determined; UR= unrelated*

- Phase I open label, 2 -step dose escalation study of NOX66, a suppository formulation, in patients with refractory solid tumours.
- Tumours selected for 5 phenotypes: breast, head and neck, lung, prostate and ovarian.
- Total 16 evaluable patients: n=8 allocated to 400mg NOX66 dosage Cohort 1; n=8 allocated to 800mg dosage Cohort
- The study comprises 2 stages of assessment:
  1. Monotherapy: NOX66 is administered for daily for 14 consecutive days
     - plasma and urine samples collected throughout
  2. Combination therapy: NOX66 plus IV Carboplatin
     - Up to 6 x 28 day cycles; NOX66 Days 1-7, Carboplatin Day 2
     - Cycles 1-3 = Low Dose (AUC4); Cycles 4-6 = Standard Dose (AUC6)
- Patient assessed for safety parameters
- Preliminary response on CT images by investigator per RECIST 1.1 at 3 months (Cycle 3) and 6 months (end cycle 6)
- Replacement of non-evaluable patients is permitted

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