ENOX2 levels of patients receiving NOX66

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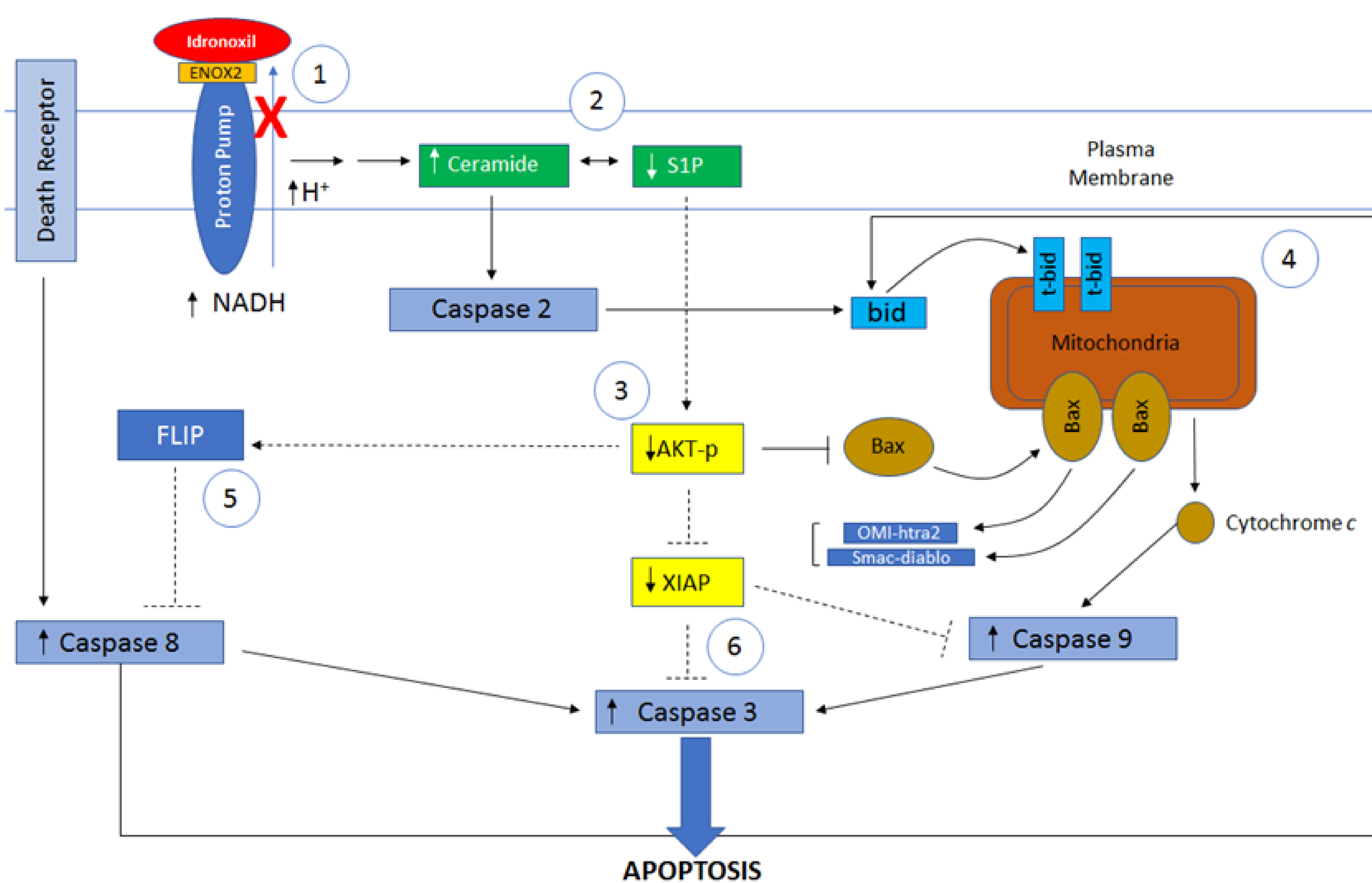
INTRODUCTION

The ECTO-NOX, or ENOX, proteins are a family of NAD(P)H oxidase proteins present on the cell surface of plants and animals. These proteins have two known and distinct biochemical activities, namely hydroquinone (NAD(P)H) oxidation and protein disulphide-thiol interchange, and are believed to play an important role in cell growth. In its constitutive form (CNOX, or ENOX1) these activities oscillate consistently at 24 min intervals. A second form of ENOX (tNOX, or ENOX2) has been identified, which has been found to be expressed on tumour cell surface and detected in sera of cancer patients[1]. Whilst ENOX2 performs the same dual function as ENOX1, the oscillation between functions occurs at 22 min intervals. From this, it is hypothesised that ENOX2 represents an important role in tumour cell growth and proliferation[2].

Importantly, while ENOX1 is refractory to quinone site inhibitors, the activity of ENOX2 may be suppressed by such inhibitors. It is this property which has led to investigation of ENOX2 inhibition as a target for anti-cancer therapy and the development of the isoflavone compound Idronoxil as a first-in-class inhibitor of ENOX2. The imputed pathway of ENOX2, and the mechanism by which inhibition of ENOX2 by Idronoxil may directly (via apoptosis) and indirectly (in combination with chemotherapy and / or radiotherapy via inhibition of DNA repair mechanisms) is outlined in Figure 1.

The compound NOX66, is a novel formulation and delivery mechanism for Idronoxil and is under clinical investigation for use in combination with standard chemotherapy and radiotherapy. The first-in-human study of NOX66, as a monotherapy (for safety evaluation) and in combination with carboplatin, is currently ongoing. Sixteen (16) patients with late stage metastatic disease (of primary origin prostate, lung, breast or ovarian) receive one of two doses of NOX66 (400mg, 800mg) as monotherapy (for 14 consecutive days) followed by low dose (AUC4) carboplatin for 3 x 28-day cycles and standard dose (AUC6) carboplatin for 3 x 28-day cycles. This poster presents interim results for the analysis of plasma ENOX2 levels in this cohort of patients.

Figure 1. Putative biochemical pathway associated with idronoxil.



The cascade of events outlined above is as follows[2-5]:

1. Idronoxil binds to ENOX2, leading to inhibition of the trans membrane electron pump which, in turn, leads to an accumulation of proton ions within the plasma membrane.
2. Accumulation of protons disrupts sphingomyelin pathway with blockage of ceramide conversion to S1P – leading to a decrease in S1P and an increase in Ceramide within the plasma membrane.
3. Decrease of S1P leads to a reduction in PI3K, Akt and XIAP and an increase in Caspase 2.
4. Reduction in Akt leads to reduction in NF-κB and allows up regulation of the intrinsic (mitochondrial) pathway of apoptosis, via an increase in Caspase 9 and Caspase 3, leading to cell death.
5. Reduction in Akt also results in an inhibition of FLIP resulting in an increase in Caspase 8 (activated via the Death Receptor on the Protein Membrane) – leading directly and indirectly (via the intrinsic pathway) to an increase in Caspase 3 and apoptosis.
6. Reduction in XIAP prevents down regulation of Caspase 9 and Caspase 3, supporting apoptosis.

METHODS

Samples for analysis were collected from patients participating in the study "NOX66-001: Safety, PK and Efficacy of NOX66 as a Monotherapy and Combined with Carboplatin in Refractory Solid Tumours" (ClinicalTrials.gov identifier NCT02941523). Commercially available lung and prostate tumour plasma controls were used as reference for qualitative review and non-tumour plasma was used as negative control.

Determination of plasma ENOX2 levels was conducted at Crux Biolab, Melbourne, Victoria, using the Human Ecto-NOX Disulfide-Thiol Exchanger 2 (ENOX2) ELISA Assay. This assay employs a quantitative sandwich linked immunoassay sorbent procedure with microplates pre-coated with an antibody specific to ENOX2. Following exposure of a sample to the antibody, any unbound substance is removed and a biotin-conjugated antibody (specific for ENOX2) is added. Avidin-enzyme conjugated horseradish peroxidase is introduced, followed by a substrate solution initiating colour development then an acid based solution is used to stop the reaction. Colour intensity is measured and correlated to ENOX2 concentration (pg/mL). In order to assess samples within the range of the assay, control samples were diluted 5-10-fold and patient samples analysed at 5-fold dilution. All samples were analysed in duplicate.

RESULTS

Figure 2. ENOX2 levels of control tumour plasma samples (measured by 5x and 10x dilution)

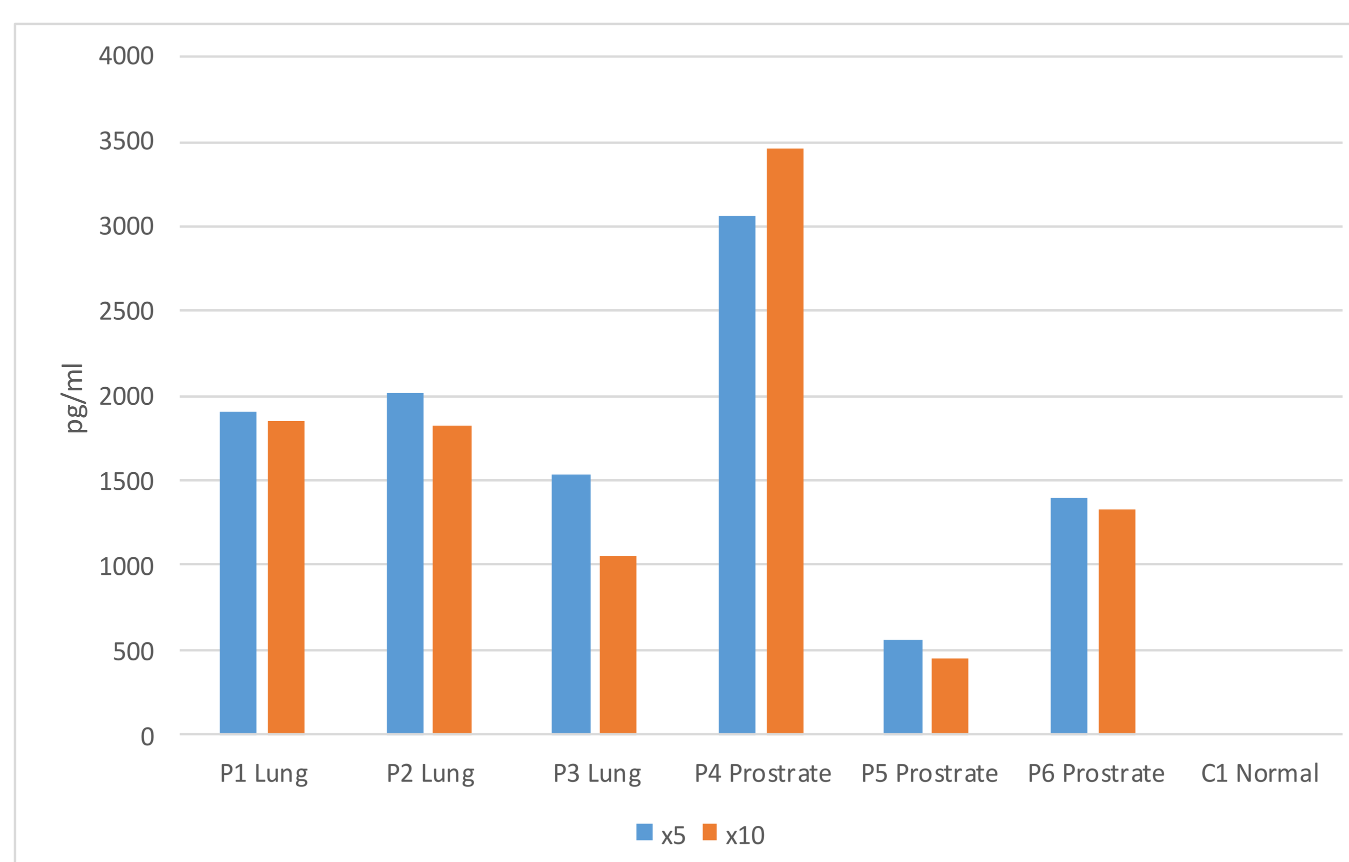
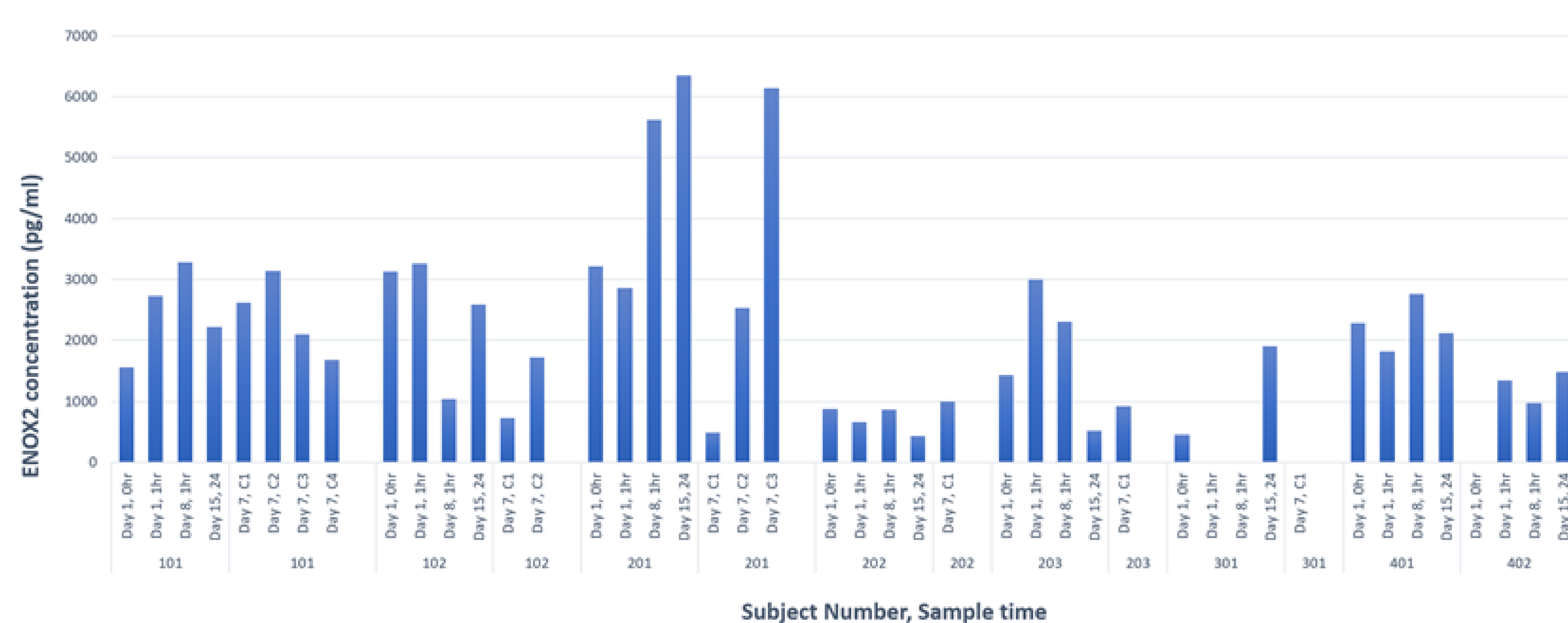


Figure 3. ENOX2 levels of patient samples, NOX66-001 study



CONCLUSION

The human ENOX2 ELISA assay allows for the measurement of ENOX2 in human plasma samples.

Intra-patient comparison of plasma ENOX2 shows variation in concentrations between samples, suggesting that shedding of ENOX2 protein may not remain consistent and indirect measurement of ENOX2 via plasma concentrations may not provide a quantitative assessment of ENOX2 activity.

Further validation of the ELISA based ENOX2 assay, and direct analysis of tumours (e.g. via biopsy) are required to allow relationship of ENOX2 level and efficacy of NOX66 to be assessed.

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