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Sinclair Nanopig™: From Multi-Omics Characterization to Pharmacology and Toxicology Validation: Underline Drug Metabolism and Immune System

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INTRODUCTION

Sinclair Nanopig™ was recently introduced by Altasciences as the next-generation nonrodent model for (bio)pharmaceuticals safety assessment. We have generated and reported physiologic and toxicologic reference values of Nanopig™ including limited growth rate and lower body weight, similar clinical pathology data, organ weights, and background microscopic findings to other minipig breeds (SOT 2023).

Genome-based comparison of drug targets together with quantitative tissue protein expression analysis enable the systematic comparison of orthologous sequences of therapeutic target (DNA or protein) and allows rational prediction of pharmacology, cross-reactivity, and potential toxicity of human drugs in animal models therefore improving clinical translation and drug attrition.

OBJECTIVE

With increasing interest/demand for using Nanopig™ in drug development from (bio)pharmaceutical industries, and in consideration of the 4R principle, this study aimed to further **provide genomic, proteomic, and functional characterization data of Nanopig™ as scientific justifications for human-relevant animal species selection** to support regulatory pharmacology and drug safety assessment**,** as well as **expanding translational knowledge in Nanopig™ to reduce and replace traditional non-rodent models in drug development.**

METHODS

Altasciences and University of Missouri Collaboration

All-in-One Platform

Nanopig™ whole genome sequencing (WGS)
- MU genomics technology core (Illumina® NGS service provider)

illumina NovaSeq 6000 — The next era in sequencing starts now

Nanopig™ tissue (15) proteome
- MU Charles W Gehrke Proteomics Center (Evosep HPLC - Bruker Mass Spectrometry[2])

EVOSEP ONE - BRUKER timsTOF Pro2

Nanopig™ genomic and proteomic data analysis (vs. reference pig and human)
- MU bioinformatics and analytics core
- Integrate multi-omics offers a holistic view (connecting genotype to phenotype)
- Better understanding of normal development, drug target homology, safety signals, and protein biomarkers discovery

Whole Genome Sequencing
- Six Nanopigs™ (3M and 3F; 4 months old) from Sinclair Bio Resources were used to isolate gDNA (Qiagen DNeasy Blood and Tissue Kit #69504) from circulating white blood cells (WBCs).
- gDNA concentration (Invitrogen Qubit #Q33230) and integrity (100 ng on 1% agarose gel) assessed/ for library preparation (Illumina #20060059; 500 ng gDNA; target insert size 550 bp).
- All six library samples (8.75-13.2 ng/µL with fragment size 691-745 bp) were sequenced on NovaSeq 6000 PE150 flowcell (Illumina's NGS protocol) with a 45-fold depth of coverage.

Bioinformatics
- Paired-end sequencing methods were applied, and clean reads were mapped to the reference Duroc pig (Sscrofa11.1; Ensembl Size 2,501,912,388 bp) for the Nanopig™ genome assembly.
- Nanopigs™ genome annotation was performed to determine the similarities and differences among the reference Duroc pig and human (GRCh38.p14; Ensembl Size 3,099,750,718 bp) databases, underline metabolism and immune systems (relevance to the drug or biologics safety assessment).

Proteomics
- Selected 15 tissues (with pharmaceutical relevance) collected from Nanopigs™ (1M and 1F; 4 months old).
- Tissue-extracted protein (20 µg) digested with Lys-C and trypsin urea buffer and fractionated peptides (500 ng/sample) acquired using DIA-PASEF to create a spectral library.
- All generated peptide library samples analyzed by LC-MS/MS for sequence data acquisition.
- Spectronaut (18.3) and MSStat (R) used for protein identification and relative label-free quantification against reference pig (UniProt-UP000008227; 46,179 protein count) and human (Uniprot-UP000005640; 20,381 protein counts) protein sequence databases.

RESULTS

Nanopig™ Genome vs. Reference Duroc Pig and Human: Similarity and Difference

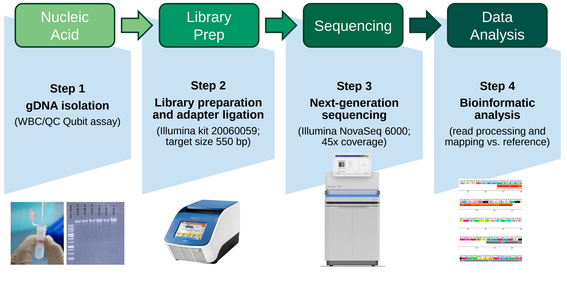

Figure 1. Nanopig™ whole genome sequencing workflow

Nanopig™ Genomic Sequences Compared With Reference Genome of Duroc Pig (NCBI sscrofa11.1)

- Nanopig™ whole genome sequencing data was obtained (for the first time) in high quality.
- Nanopig™ genome assembled at chromosome level with a total length of ~**2.9 Gb** (vs. Duroc pig 2.5 Gb).
- Alignment and mapping coverage of sequences **>98%** with no clear and substantial genomic variance (**Figure 2a**).
- Variant annotation indicates male and female Nanopigs™ replicates are consistent and have high repeatability (**Figure 2b**).
- These results indicate distinct characteristics of Nanopigs™ derive from **small-scale alterations in the genome (single nucleotide polymorphisms or translational modifications)**, rather than large-scale deletion or insertion polymorphisms.

No.	Sample	Total mapped
1	25625-M	825,864,920 (98.47%)
2	25626-M	851,289,469 (98.47%)
3	25627-M	975,456,368 (98.36%)
4	25634-F	866,940,025 (98.24%)
5	25635-F	856,232,831 (98.24%)
6	25636-F	882,552,801 (98.42%)

Figure 2a. Mapping coverage of sequences

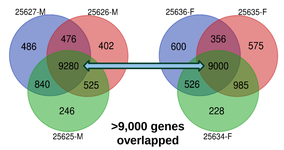

Figure 2b. Variant annotation (male vs. female)

Nanopig™ genomic sequences compared with reference genome of Human (NCBI GRCh38.p14)

- Nanopig™ genome assembled at chromosome level with a total length of ~**2.9 Gb similar to the human genome (3.1 Gb).**
- To further elucidate the genetic basis of Nanopig™ metabolism and immune systems.

Human comparative genomic analyses (gene homology) revealed **1,606 immunity-related genes and 11,711 metabolism-related genes overlapped with Nanopig™.**

- Cytochrome P450 (CYPs) are known as crucial and responsible for most drug metabolism in humans.
- Specifically, a total of 47 CYP450 genes identified in Nanopig™ with **20 in the CYP family 1/2/3 similar to Human 57 CYP450 genes with 24 in CYP1/2/3.**

Nanopig™ Tissue Proteomics: Human Orthologous Gene Expression

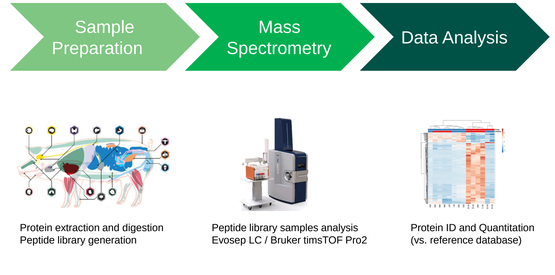

Figure 3. Nanopig™ proteomics workflow

Nanopig™ Proteomic Data Compared With Reference Human Proteome Database (Uniprot-UP000005640)

- Nanopig™ proteins expression profile (function of genes) successfully achieved in 14 major tissue and blood samples.
- These proteomic data are critical because many cellular mechanisms depend on post-translational modification of proteins and specific drug-protein interactions, especially in drug metabolism and biologics-induced immunogenicity and/or immunotoxicity.
- **Drug metabolism enzymes and transporters (DMET)** play an essential role in drug disposition, human orthologous of Phase I/Phase II enzymes and transporters (influx/efflux) identified in Nanopig™:

Liver	44 Phase I, 27 Phase II enzymes, and 96 transporters
Small Intestine	18 Phase I, 17 Phase II enzymes, and 116 transporters
Kidney	33 Phase I, 21 Phase II enzymes, and 152 transporters

- As there are many similarities in metabolic enzymes and transporters to those in humans, **Nanopig™ is a relevant and powerful animal model for candidate drug toxicokinetic and regulatory toxicology studies.**

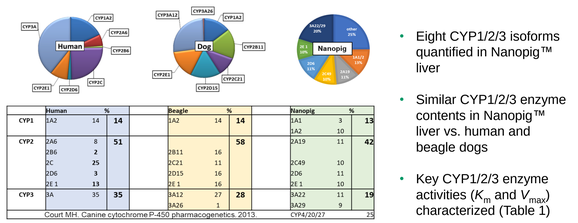

Figure 4. Nanopig™ vs. human and beagle dog liver CYP1/2/3 abundance

- Eight CYP1/2/3 isoforms quantified in Nanopig™ liver
- Similar CYP1/2/3 enzyme contents in Nanopig™ liver vs. human and beagle dogs
- Key CYP1/2/3 enzyme activities (K_m and V_{max}) characterized (Table 1)
- **Immune system components and effectors** (innate and adaptive) human orthologous profiled for biologics testing in Nanopig™:

Plasma	30 antimicrobial, 11 APP, 25 cytokines, C3, C5, C8, and IgM
Thymus	78 antimicrobial, 54 APP, 46 cytokines, 47 TCR and 35 BCR signaling
Spleen	92 antimicrobial, 51 APP, 51 cytokines, 50 TCR and 40 BCR signaling
LN (cervical)	94 antimicrobial, 52 APP, 60 cytokines, 55 TCR and 45 BCR signaling

- Swine leukocyte antigens (SLAs) identified in each Nanopig™ are critical for immunological reactions. Further assessment of the utility of Nanopig™ for immunosafety testing using reference biologics (tested preclinically and clinically) is warranted.

Nanopig™ Hepatic Key CYP450 Enzymes Activity Assay (BioIVT)

Table 1: Experimental conditions and determination of kinetic constants (K_m and V_{max})

Probe Substrate	Enzyme Subfamily	Final Protein Conc. (mg/mL)	Incubation Time (min)	Concentration Range (µM; 13 concentrations)
Phenacetin	CYP1A	0.1	10	1, 2, 4, 7, 9, 11, 14, 17, 22, 28, 55, 83, 110
Diclofenac	CYP2C	0.1	40	0.5, 1, 2, 5, 10, 15, 20, 25, 31, 38, 50, 63, 125
Dextromethorphan	CYP2D	0.01	20	0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.25, 1.5, 2, 2.5, 5, 7.5, 10
Midazolam	CYP3A	0.1	10	0.2, 0.4, 0.8, 1.2, 1.6, 2, 2.5, 3, 4, 5, 10, 15, 20

Probe Substrate	Enzyme Subfamily	Human K_m	Human V_{max}	Nanopig™ K_m	Nanopig™ V_{max}	Canine K_m	Canine V_{max}
Phenacetin	CYP1A	94.5 ± 3.8	662 ± 13	4.23 ± 0.56	988 ± 45	54 ± 30	790 ± 250
Diclofenac	CYP2C	9.16 ± 0.33	2610 ± 30	7.89 ± 1.15	91.4 ± 5.3	29 ± 8	990 ± 30
Dextromethorphan	CYP2D	11.0 ± 0.9	225 ± 6	0.811 ± 0.070	879 ± 23	0.72 ± 0.16	2600 ± 100
Midazolam	CYP3A	2.52 ± 0.19	807 ± 18	4.06 ± 0.41	621 ± 39	1.5 ± 0.5	270 ± 50

BioIVT study report reference XT234148; K_m: µM; V_{max}: pmol/min/nmol total P450 or mg protein; Mean ± SD

Correlation analysis between Nanopig™ hepatic CYP protein abundance and enzymatic activity investigated with the most commonly used probe substrates (*FDA guideline: Drug Development and Drug Interactions | Table of Substrates, Inhibitors and Inducers*). The K_m values for the CYP2C substrate diclofenac and the CYP3A substrate midazolam were most similar between Nanopig™ and human (CYP2D: most similar between Nanopig™ and canine; CYP1A: most similar between human and canine).

Nanopig™ Immunophenotyping, Cytokines, and Function Evaluation (T-dependent antibody responses; TDAR)

Strategy and preliminary data for characterization of the Nanopig™ immune system (cellular, humoral, and functional assessment):

- **Blood lymphocyte subset:** identify B lymphocytes, natural killer cells, NKT cells, αβ- and γδ-T lymphocytes in Sinclair and other breeds of minipigs PBMCs (SOT 2018).
- **Serum cytokines (Luminex porcine 13-plex)**: identify multiplex cytokines panel and confirmation with ELISA in serum of Sinclair and other breeds of minipigs.
- **TDAR (immunosuppression and/or immunostimulation) assay** (under planning) to keyhole limpet hemocyanin (KLH) in the Nanopig™ (serum IgM and IgG responses).

SUMMARY AND CONCLUSION

- This is the first report of a newly revealed chromosome-level-based version of the Nanopig™ genome together with a comparative human orthologous proteomics in tissues (pharmaceutical relevance) underline metabolism and immune system as the basis for translational research.
- Functional genomics and proteomics in Nanopig™ are of great importance for developing and characterizing Nanopig™ used in drug and biologics development, which allows an understanding of the complex molecular mechanisms that control the biology and pathology.
- Our searchable multi-omics database will encourage the broad use of the Nanopig™ for pharmacology, biomarkers discovery, and drug safety assessment.

Reference:
Bendixen E, et al. Advances in porcine genomics and proteomics--a toolbox for developing the pig as a model organism for molecular biomedical research. Brief Funct Genomics. 2010 May;9(3):208-19.
Heckel T, et al. Functional analysis and transcriptional output of the Göttingen minipig genome. BMC Genomics. 2015 Nov 14;16:932.
Helke KL, et al. Pigs in Toxicology: Breed Differences in Metabolism and Background Findings. Toxicol Pathol. 2016 Jun;44(4):575-90.
Schelstraete W, et al. Characterization of Porcine Hepatic and Intestinal Drug Metabolizing CYP450: Comparison with Human Orthologues from A Quantitative, Activity and Selectivity Perspective. Sci Rep. 2019 Jun 25;9(1):9233.
Buyssens L, et al. Hepatic Cytochrome P450 Abundance and Activity in the Developing and Adult Göttingen Minipig: Pivotal Data for PBPK Modeling. Front Pharmacol. 2021 Apr 15;12:665644.
Arbitrio M, et al. DMET™ (Drug Metabolism Enzymes and Transporters): a pharmacogenomic platform for precision medicine. Oncotarget. 2016 Aug 16;7(33):54028-54050.
Rubic-Schneider T, et al. Minipigs in Translational Immunosafety Sciences: A Perspective. Toxicol Pathol. 2016 Apr;44(3):315-24.