

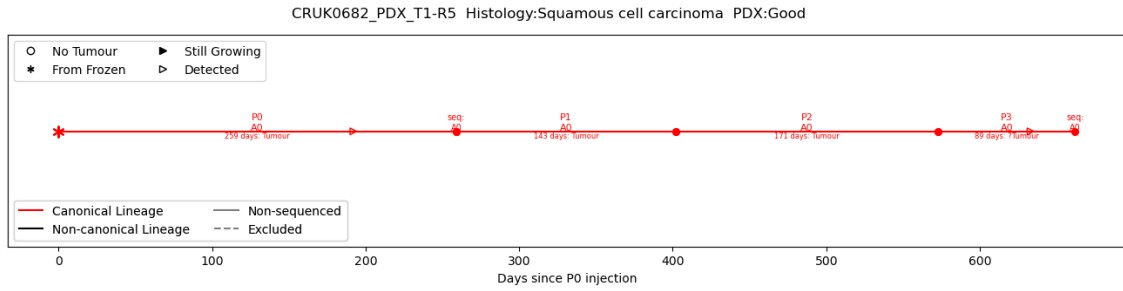
## NSCLC PDX Information Sheet

CRUK0682\_PDX\_T1-R5

**Lung TRACERx patient ID:** CRUK0682**Patient tumour number:** T1**Primary tumour region:** R5**Primary histology:** Lung Squamous Cell Carcinoma**Driver mutations:** KEAP1 (clonal - KEAP1:NM\_012289:exon6:c.A1715G:p.Y572C)  
TP53 (clonal - TP53:)

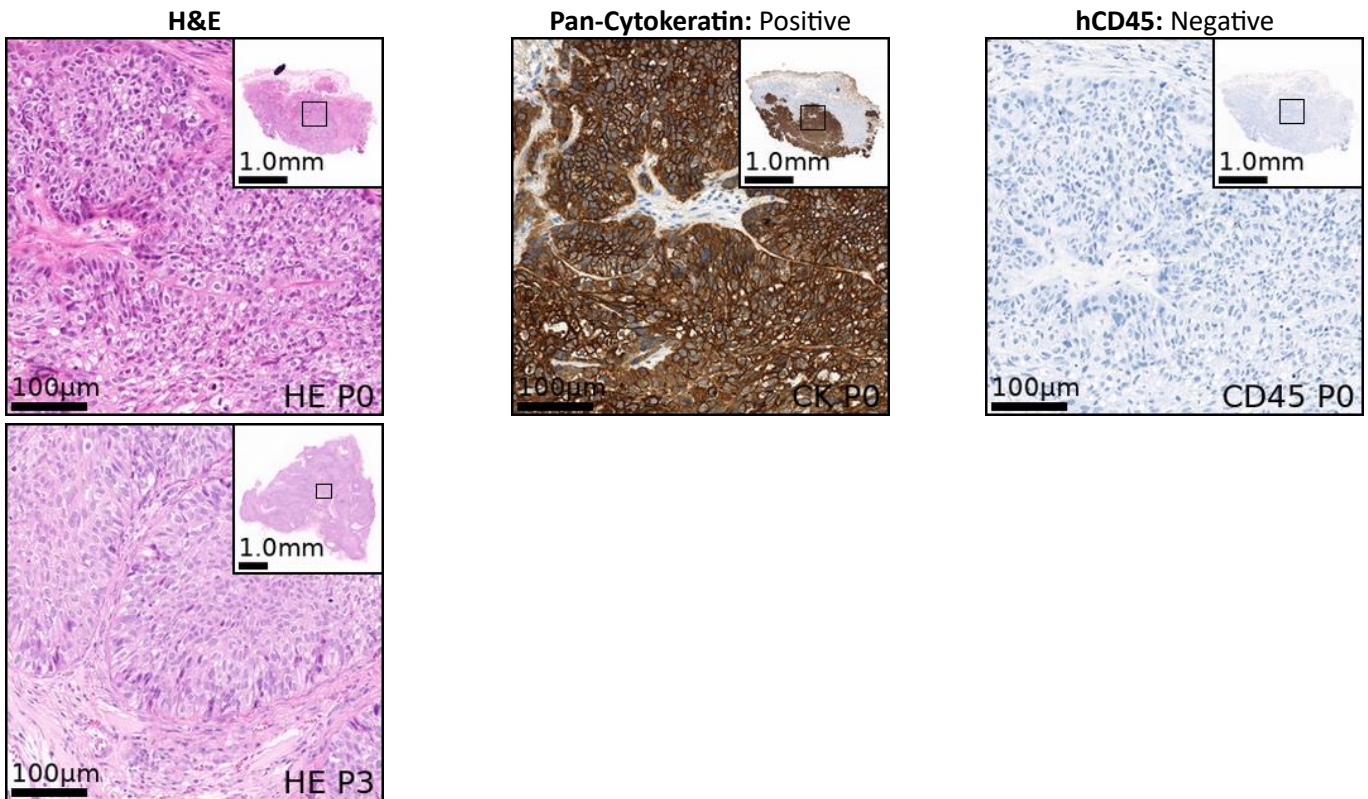
Mutation calls for PDX models and primary tumour regions are available via Hynds RE, Huebner A, Pearce DR, et al. (2024). Only driver mutations found in primary tumour and PDX or in P0 and P3 PDX are shown. Where PDX model sequencing is not available driver mutations shown are those found across all regions of the primary tumour.

**Lot:****Passage:****Model notes:****Patient****Sex:** Male**Age:** 73 years**Ethnicity (self-reported):** White- British**Smoking status:** Ex-Smoker**Neoadjuvant treatment:** None. Lung TRACERx study criteria exclude neoadjuvant treatment.**Tumour****Resection site:** Lung, Primary NSCLC surgery**T stage (v8):** 1c**N stage (v8):** 0**TNM stage (v8):** 1a**Lesion size:** 30 mm**Xenograft model****Host strain:** NOD scid gamma (NSG, Charles River Laboratories)**Host site:** Subcutaneous (flank)**Injection vector:** Matrigel®, growth factor reduced**Host sex:** Male**Sample preparation:** Minced**Time to harvest P0:** 259 days**Time to harvest P1+:** 143, 171, 89 days



Figures generated with [github.com/EpiCENTR-Lab/PDX-Tracker](https://github.com/EpiCENTR-Lab/PDX-Tracker)

## Immunohistochemistry



Figures created with PATHOverview: [github.com/EpiCENTR-Lab/PATHOverview](https://github.com/EpiCENTR-Lab/PATHOverview)

## PDX model methods

### Materials

Transport medium: MEM alpha medium (Gibco) containing 1X penicillin/streptomycin (Gibco), 1X gentamicin (Gibco) and 1X amphotericin B (Fisher Scientific, UK)

Freezing medium: foetal bovine serum with 10% DMSO

Growth factor-reduced Matrigel (BD Biosciences)

16G needle and 1ml low dead-volume syringe

### Preparation of sample from frozen

Warm the cryovial in a 37°C water bath until just thawed. Transfer the whole sample to a sterile 1.5 ml centrifuge tube and top up with transport medium. Centrifuge at 300 g for 5 min at 4°C and remove the supernatant. Wash sample with transport medium. If required, collect any large pieces of tissue and finely mince with a scalpel to ensure they will pass through the needle. Centrifuge the sample and gently resuspend in 180 µl ice-cold Matrigel, keep the sample on ice before subcutaneous injection.

### Sample injection

All animal procedures must be performed in accordance with animal licence conditions.

Anesthetise the host with 2–4% isoflurane and shave and clean the flank. Injection should be performed without delay to ensure Matrigel does not solidify in the syringe. Inject the sample subcutaneously with a 16 G needle in one bolus. Pause to allow Matrigel to begin to solidify before needle removal.

When xenograft tumours have formed, tumour measurements should be taken in two dimensions using callipers and mice euthanized before tumour size reaches licence limits. (In our study this was 1.5 cm<sup>3</sup> in volume. Volume was calculated as 0.5 x length x width<sup>2</sup>).

### Passage

Cull mice using an approved method. In our study, mice were culled in an increasing concentration of CO<sub>2</sub> followed by cervical dislocation. Spray the xenograft site with 70% ethanol to damp the fur and aseptically isolate the xenograft while removing any visible mouse subcutaneous fat / stroma. Take approximately 1/8<sup>th</sup> of the xenograft, finely mince and resuspended in 180 µl ice-cold Matrigel for subcutaneous injection in a new host as above. Xenograft tissue may be held in transport media on ice for a short period (up to overnight storage).

### Cryopreservation

Finely mince xenograft tissue and transfer sufficient material for one injection to a cryovial. Resuspend the sample in 0.5 – 1.0 ml ice-cold freezing medium and freeze first to –80°C in a CoolCell (Corning) before long-term storage in liquid nitrogen.

**References:****PDX generation, characterisation and exome sequencing:**

Hynds RE, Huebner A, Pearce DR, et al. (2024) Representation of genomic intratumor heterogeneity in multi-region non-small cell lung cancer patient-derived xenograft models. *Nat Commun.* 15, 4653.

<https://doi.org/10.1038/s41467-024-47547-3>

**PDX quality control:**

Pearce DR, et al. (2023) Phenotyping of lymphoproliferative tumours generated in xenografts of non-small cell lung cancer. *Front Oncol.* 13. <https://doi.org/10.3389/fonc.2023.1156743>

**Lung TRACERx study:**

<https://www.nature.com/collections/haffgaicaf>

Jamal-Hanjani M, et al. (2014) Tracking Genomic Cancer Evolution for Precision Medicine: The Lung TRACERx Study. *PLoS Biol.* 12. <https://doi.org/10.1371/journal.pbio.1001906>