Frederick National Laboratory for Cancer Research

Method Development for Generation of PDX models from Rapid Autopsy Samples for the NCI Patient-Derived Models Repository

Yvonne A. Evrard[1]*, Michelle A. Eugeni[2]*, Michelle Ahalt-Gottholm[2], Carrie Bonomi[1], Suzanne Borgel[1], Thomas C. Caffrey[3], John Carter[1], Ting-Chia Chang[1], Li Chen[1], Kevin Cooper[1], Biswajit Das[1], Emily Delaney[1], Kelly Dougherty[1], Eleonora Duregon4§, Stephanie Ecker[1], Joseph Geraghty1, Marion Gibson[1], Lauren Hicks[1], Jenna Hull[1], Sharon Int Veldt[1], Shahanawaz Jiwani[1], Chris A. Karlovich[1], Jade Loewenstein[1], Candace Mallow[1], Chelsea McGlynn[1], Justine Mills[1], Tiffanie Miner[1], Jowaly Schneider[4], Tia Shearer[1], Savanna Styers[1], Shannon Uzelac[1], Paul Grandgenett[3], Michael Hollingsworth[3], Jody E. Hooper*4†, P. Mickey Williams[1], Melinda G. Hollingshead[5], James H. Doroshow[5].

Affiliations: [1]Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, MD, [2]Biological Testing Branch, Developmental Therapeutics Program, National Cancer Institute at Frederick, Frederick, MD; [3]University of Nebraska Medical Center; [4]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD; and [5]National Cancer Institute, Division of Cancer Treatment and Diagnosis, Bethesda, MD

*Co first authors; †Current position: Stanford University School of Medicine, Stanford CA; §Current position: National Institute on Aging, National Institutes of Health

Contact Yvonne A. Evrard:* yvonne.evrard@nih.gov

Poster #4669

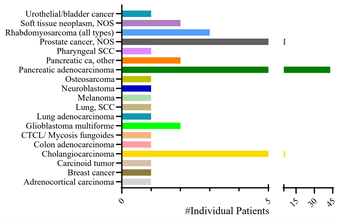
Patient Characteristics for Method Development

NCI's Patient-Derived Models Repository (NCI PDMR; https://pdmr.cancer.gov) has a variety of patient-derived models available across most solid tumor histologies. These models are early passage, genetically characterized and associated with limited patient treatment history. As part of this effort, the NCI PDMR and the Biological Testing Branch (BTB, DCTD, NCI) worked with the University of Nebraska Medical Center (UNMC) Rapid Autopsy Program (RAP) and Johns Hopkins University (JHU) Legacy Gift Rapid Autopsy Program to develop and optimize methods for collection, processing, and shipping of autopsy tumor material to maintain viability during overnight transit for use in patient-derived model development. These methods have been successfully transferred to two other participating rapid autopsy programs.

As of November 2021, 412 autopsy tumor samples from 76 consented patients have been received for model development; 348 shipped overnight in media for next day implantation into NSG host mice and 64 cryopreserved prior to shipping for a comparative assessment of take-rate versus fresh tumor samples. On average 3-8 tumor samples, primary and metastatic, were collected post-mortem from the truncal region of each patient. Histologies include Pancreatic adenocarcinoma (n=43 patients), Cholangiocarcinoma (n=6), Prostate adenocarcinoma (n=6), and 21 others with 1-2 patients/histology. The overall age range of enrolled patients at time of collection was 5-88yo. The post-mortem interval for collections ranged from 1.5 to 20 hours with a median of 3h (avg. 3.75h; outlier >11h removed). Collection methods were optimized to reduce contamination and increase viability of tumor tissues for successful PDX model generation.

- **Breakout by Age and Gender**
  - Female
    - Total individual patients: 24
    - Age range at diagnosis: 37-85 yo
      - Average age: 68.3 yo
      - Median age: 71 yo
  - Male
    - Total individual patients: 53
    - Age range at diagnosis: <1-82 yo
      - Average age (pediatric omitted): 62.1 yo
      - Median age (pediatric omitted): 64 yo
    - All pediatric collections to date have been from male patients. Ages range from <1-13 yo at diagnosis (5-18 yo at time of collection).

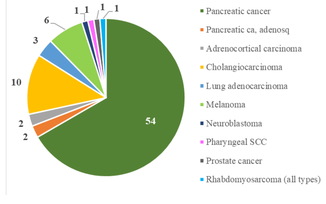
Patients Enrolled by Histology



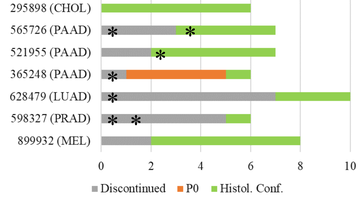
Successful Models Developed to Date

Of 348 fresh tumor samples collected (thru Nov 2021), 69 PDX models from 33 patients had been generated (range 1-6 models/patient) and an additional 55 samples are being monitored for growth in passage[2]. The largest public single-patient PDX model sets are for melanoma (899932-113-R, n=6) and two pancreatic adenocarcinomas (521955-158-R, n=6, 217524-143-R, n=4). The following graphs show the number of histologically confirmed PDXs by histology and examples of take rates for 7 different patient models sets. The asterisks (*) in the take rate graph indicate take-rates for primary tumor material, the remaining samples were from metastatic tumor sites (some patients had tumor material from more than one lesion at the primary site of diagnosis.

Histopathologic Confirmed PDX Models



Take Rate Examples



Method Development

Post-mortem interval

- Multiple factors contribute to the length of the post-mortem interval
  - Location of patient death – inpatient, outpatient hospice, etc.
  - Availability of staff to conduct the post-mortem, this will vary by institution for a variety of reasons, such as availability of on-call staff versus sites with limited facility access after hours
  - Regulations regarding disposition of the body vary by state
    - For example, family consent may still be required post-mortem even if the patient consented pre-mortem
  - Allowing time for families to be with the patient post-mortem.
- Controllable factors to minimize post-mortem interval
  - Establish a specific catchment range so time in transport is minimized for those that are outpatient.
  - Coordinate plans for patient transport with a dedicated transport service.
  - Involve the family in discussions, per patient wishes, prior to the patient death
  - Establish a dedicated on-call team
  - Build a relationship with both inpatient and outpatient hospice care to allow for streamlined communication (per patient agreement/consent regulations).

Post-mortem Interval Range and Take-Rate for Histologically Confirmed PDXs

Overall percent take-rate is similar to what is observed with fresh tissue collections across solid tumor histologies

Post-Mortem Interval (hrs)	Number of Specimens Received	PDX Pathology Confirmed
1.5 – 5.0	360	167 (46%)
>5.0 – 10.0	56	18 (32%)
>10.0	8	2 (25%)

Mediation of bacterial and fungal contamination

Initial rapid autopsy/post-mortem specimen collections involved direct placement of the tumor tissue into CO_2-independent media containing a mix of antibiotic and antifungal agents. Specimens were shipped overnight for arrival the next day for model development by the Biological Testing Branch (BTB, DCTD, NCI). Most samples were received within 24 hrs post-collection, a smaller subset was received 48-96 hours post-collection. Upon receipt, specimens are rapidly processed with a portion of the received tissue cultured in vitro to develop patient-derived cell lines and an aliquot of the transport media is cultured to test for sterility. The remaining tissue was used for in vivo implantation[2].

Early in vitro culture results demonstrated frequent presence of extensive bacterial contamination including but not limited to those listed below. Fungal contamination was also a frequent occurrence. In vivo, all animals receive antibiotic feed for approximately 2 weeks post-implant. Despite the confirmed presence of the below contaminants, minimal overt animal toxicity was observed; though in vitro models were lost.

- *Pseudomonas aeruginosa*
- *Enterococcus faecalis*
- *Staphylococcus epidermidis and intermedius*
- *Lysinibacillus sphaericus*
- *Bosea massiliensis*

Based on this initial experience, it was determined that preliminary processing of the resected tumor tissue prior to placement in the sterile transport media would be necessary to mediate contaminants that may be present on the external surface of the collected tumor tissue. In coordination with the Biological Testing Branch (DTP, DCTD, NCI-Frederick) and the UNMC and JHU RAP teams, procedures to (1) process the resected tissue through a series of H_2O_2 dips and rinses to remove an adequate amount of the surface contaminants with minimal impact to the deeper tissue cell viability and (2) increase overall sterile technique for research autopsy/post-mortem tumor collections were implemented. The now established SOPs are publicly available on the NCI PDMR website[1].

Important methods for reducing contaminants during post-mortem tumor collection included:

- Cleaning the exterior of the body or at least the incision line area prior to first incision
- Use of separate sets of sterile instruments for initial external and internal incisions (Rib shears/saw cannot be autoclaved)
- Separate set of disposable sterile instruments for each unique tumor location
- Use of 'production line-like' process for H_2O_2 dip & rinsing of resected tissue
- Use of mobile cart with sterile drape as a work surface

References

1. SOPs for collection and shipping of tumor material: https://pdmr.cancer.gov/sops/default.htm#h01
2. SOP for implantation of patient material for PDX development: https://pdmr.cancer.gov/content/docs/SOP50101_Tumor_Implantation_PDX.pdf
3. Chakravarty et al. OncoKB: A Precision Oncology Knowledge Base (2017), 10.1200/PO.17.00011

Funded by NCI Contract No. HHSN261200800001E

Single-patient PDX Model Sets

Three representative rapid autopsy model sets are shown below including: synopsis of patient diagnosis and tumor material collected, oncogenic and likely oncogenic variants as annotated by OncoKB[3] , and SNP ideograms of successfully generated PDXs. Genomic characterization was done on 4-6 PDX samples for each model across multiple passages. Within an individual model, all OncoKB variants were seen in all sequenced PDX tumors.

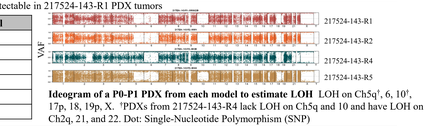
217524-143-R*n* PDX Model Set

Pancreatic adenocarcinoma (mucinous), Male, 50yo
Multiple prior therapies, MSI-Stable

217524 Model Set	Hugo Symbol	HGVS Protein Change	Avg. VAF	SD VAF
OncoKB Variants (n=23)	ARID1A*	p.E38Gfs*72	0.3888	N/A
	CDKN1B*	p.N124Lfs*20	0.3299	N/A
	KRAS	p.G12R	0.4747	0.0733
	SMAD4	p.G247Dfs*89	0.9905	0.0035
	STK11	p.S299Lfs*19	0.9901	0.0074

*Only detectable in 217524-143-R1 PDX tumors

Model ID	Resection site	Type	PDX Model
217524-143-R1	Pancreas	Primary	Yes
217524-143-R2	Liver	Metastatic	Yes
217524-143-R3	Diaphragm	Metastatic	No Growth
217524-143-R4	Pancreas [dd]	Primary	Yes
217524-143-R5	Liver [A]	Metastatic	Yes
217524-143-R6	Diaphragm [xx]	Metastatic	No Growth



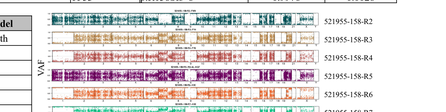
Ideogram of a P0-P1 PDX from each model to estimate LOH LOH on Ch5q[1], 6, 10[1], 17p, 18, 19p, X. [1]PDXs from 217524-143-R4 lack LOH on Ch5q and 10 and have LOH on Ch2q, 21, and 22. Dot: Single-Nucleotide Polymorphism (SNP)

521955-158-R*n* PDX Model Set

Pancreatic adenocarcinoma, Female, 60yo
Multiple prior therapies. MSI-Stable

521955 Model Set	Hugo Symbol	HGVS Protein Change	Avg. VAF	SD VAF
OncoKB Variants (n=32)	KRAS	p.G12D	0.5797	0.0255
	TP53	p.R158Sfs*8	0.9978	0.0028

Model ID	Resection site	Type	PDX Model
521955-158-R1	Tumor studding along colon	Metastatic	No Growth
521955-158-R2	Liver [A]	Metastatic	Yes
521955-158-R3	Liver [B]	Metastatic	Yes
521955-158-R4	Tumor in colonic fat	Metastatic	Yes
521955-158-R5	Pancreas	Primary	Yes
521955-158-R6	Myometrium	Metastatic	Yes
521955-158-R7	Colon	Metastatic	Yes
521955-158-R8	Diaphragm	Metastatic	No Growth



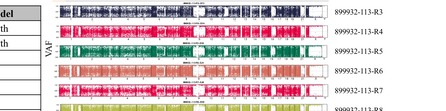
Ideogram of P0 PDXs from each model to estimate LOH . LOH on Ch1*, 9, 14, 15, 18, 19, 21, 22. *PDXs from 521955-158-R2 and R5 lack LOH on Ch1 while PDXs from 521955-158-R2 have LOH on Ch7

899932-113-R*n* PDX Model Set

Melanoma, Female, 63yo
No prior therapies, MSI-Stable

521955 Model Set	Hugo Symbol	HGVS Protein Change	Avg. VAF	SD VAF
OncoKB Variants (n=32)	KRAS	p.G12D	0.5797	0.0255
	TP53	p.R158Sfs*8	0.9978	0.0028

Model ID	Resection site	Type	PDX Model
899932-113-R1	Liver [Right - A]	Metastatic	No Growth
899932-113-R2	Diaphragm [A]	Metastatic	No Growth
899932-113-R3	Omentum [A]	Metastatic	Yes
899932-113-R4	Small bowel mesentery [A]	Metastatic	Yes
899932-113-R5	Liver [Right - B]	Metastatic	Yes
899932-113-R6	Diaphragm [B]	Metastatic	Yes
899932-113-R7	Omentum [B]	Metastatic	Yes
899932-113-R8	Small Bowel Mesentery [B]	Metastatic	Yes



Ideogram of P0 PDXs from each model to estimate LOH . LOH on Ch1p, 1q, 9, 11q, 16q, 21, X

Summary

We recommend incorporating as many of these methods as possible within the limitations of your individual site. Regulations for patient consent can differ from state to state as can staffing availability and coordination of patient transport to autopsy facilities; these as well as many other challenges need to be addressed in a site-by-site manner. It should also be noted that the methods developed for generation of models for the NCI PDMR are, in part, based on issues that are encountered due to overnight shipping of the tumor material that may not need to be addressed if the in vivo facility can receive the tumor tissue within a few hours of the post-mortem collection.

Of the 69 models developed to date, 48 are publicly available from the NCI PDMR while the rest are undergoing quality control process prior to public release including whole exome and transcriptome sequencing, short tandem repeat profiling, and full histopathologic assessment of distribution material. Models developed from autopsy material provide a research tool to investigate tumor evolution, differences between primary and metastatic lesions, and assessment of differences in therapeutic response based on differences in the tumor biology. These models will serve as a valuable resource for translational researchers interested in these and other research questions.