Quality Control Efforts in a Large-scale, Preclinical Trial of Rare Cancer PDXs by The National Cancer Institute's Patient-Derived Models Repository (PDMR)

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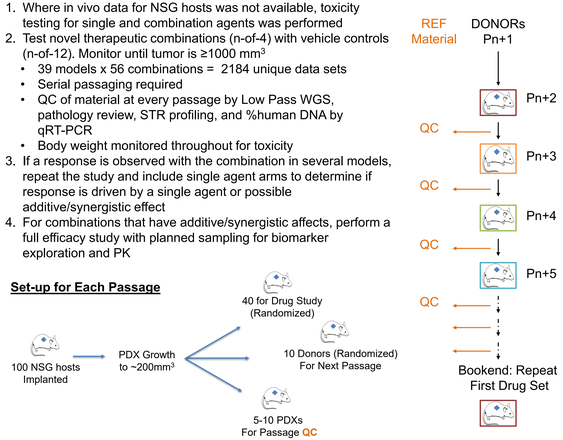
Poster #5056

Overview

The National Cancer Institute's Patient-Derived Models Repository (NCI PDMR; https://pdmr.cancer.gov) is performing a large-scale multi-year preclinical study with 39 PDX models of rare cancers (table below) treated with 56 novel therapeutic combinations in an exploratory, n-of-4 arm, study to identify novel therapeutic combinations for these underserved cancers. Combinations that show regression or durable inhibition of tumor growth are repeated along with the single agents to determine if the response is driven by the combination or one of the agents. To do this in a timely manner, the PDX tumors are serially passaged and each passage is treated with a set of 8 combinations plus relevant vehicle control(s); in parallel sufficient PDXs are retained to be expanded for the next passage and drug set. Every serial passage undergoes several quality control assessments that serve as go/no-go criteria, including pathology assessment, human:mouse DNA content assessment, and low pass whole genome sequencing to determine the average fraction of genome changed compared to the original donor material. If there is a QC failure, the PDX model is restarted from early passage cryo-material (passage 1-2). We also bookend the combination studies with the first set of agents to see if tumor response is similar across passages, a reflection of the inherent heterogeneity of the models. To date, most of the models have demonstrated a high degree of genomic, histopathologic, and response stability, though a couple of models have moved toward murine content and have been restarted from early passage. DNA and RNA are retained from all passages so a full NGS evaluation can be performed. Single agent studies of drug combinations that demonstrated a response in 30%-50% of the models conducted are also underway to determine which combinations have a more than additive effect compared to the single agents. Promising combinations will be evaluated in clinical trials at the NCI in patients with these rare cancers.

Overall Study Design

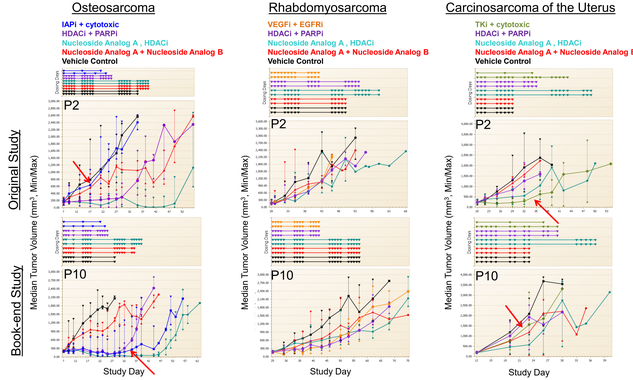
1. Where in vivo data for NSG hosts was not available, toxicity testing for single and combination agents was performed
2. Test novel therapeutic combinations (n-of-4) with vehicle controls (n-of-12). Monitor until tumor is ≥1000 mm^3
   - 39 models x 56 combinations = 2184 unique data sets
   - Serial passaging required
   - QC of material at every passage by Low Pass WGS, pathology review, STR profiling, and %human DNA by qRT-PCR
   - Body weight monitored throughout for toxicity
3. If a response is observed with the combination in several models, repeat the study and include single agent arms to determine if response is driven by a single agent or possible additive/synergistic effect
4. For combinations that have additive/synergistic affects, perform a full efficacy study with planned sampling for biomarker exploration and PK



Bookend Drug Response Comparison

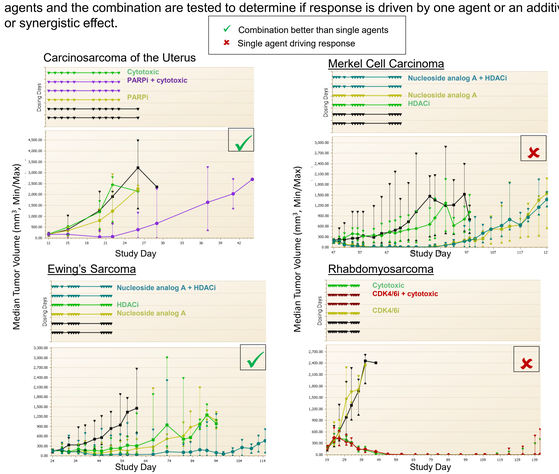
In addition to genomic and histopathologic QC assessments, a QC metric for response across passages was also implemented. Once all drug combinations have been tested, the first set of drugs tested are repeated (average: +8 passages) to determine if a higher passage tumor has an altered response compared to the initial study. These bookend studies are underway for several models.

As seen below in the first bookend studies to be completed, most therapeutic combinations have a very consistent response across passages while specific model/therapeutic combinations (red arrows) have an altered response (growth delay vs progressive growth). More studies will be needed to determine if there is a pattern between altered response across passages and genomic characteristics, mechanism of action of the therapeutics, patient clinical history, and/or histology.



Compare Single Agent to Combination

Active combinations are repeated against each single agent; examples are shown below. Single agents and the combination are tested to determine if response is driven by one agent or an additive or synergistic effect.



Rare Cancer PDX Models in Study

Diagnoses	Number of Models
Carcinosarcoma of the uterus	3
Ewing sarcoma/Peripheral PNET	3
Liposarcoma	3
Malig. periph. nerve sheath tum.	3
Merkel cell tumor	3
Neuroendocrine cancer, NOS	3
Osteosarcoma	3
Salivary gland cancer	3
Synovial sarcoma	3
Gastrointestinal stromal tumor	2
Mesothelioma	2
Rhabdomyosarcoma, NOS	2
Adenocarcinoma - anal	1
Adenocarcinoma - small intest.	1
Alveolar soft part sarcoma	1
Hurthle cell neoplasm (thyroid)	1
Penile squamous car.(epidermoid)	1
Small Cell Lung Cancer	1

Low-Pass Whole Genome Sequencing (LP-WGS) and Percent Human Tumor Content (%Hu) Assessment

Fraction of genome changed between two samples is defined as the fraction of altered genomic regions with relative copy number changes >0.4 (log2 ratio, 1.32 ploidy). Baseline distribution is determined by the average and standard deviation of fraction of genome changed from intra-model pairwise comparison of REF Material samples. For QC material, fraction of genome changed is assessed at each passage versus REF Material samples. Table represents average QC metrics for all passages where at least 4 serial passages had been assessed versus baseline REF material. Overall genome heterogeneity of models across passages is consistent with the intra-model heterogeneity observed at baseline (REF Material Only) by LP-WGS assessment.

- RT-PCR performed as described in Alcoser et al. (Biotechnology, 2011. PMID: 22176647)
- Low Pass WGS: Mouse read removal, BBSplit; Copy Number detection, CNVkit, 1 Mbp bin size

Variability of Model	Avg. %Genome Changed vs REF Material (LP-WGS)
Heterogeneous	> 5%
Intermediate Heterogeneity	1.5% ~ 5%
Homogeneous	< 1.5%

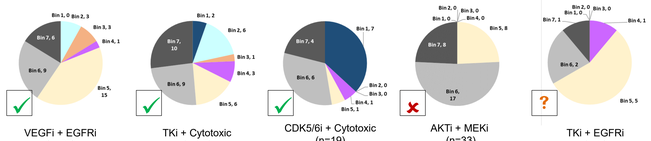
Diagnosis	REF Material Passage *	Highest Passage On-Study*	Avg %Hu (RT-PCR)	STDEV	#QC Sets Assessed	Avg. %Genome Changed vs REF Material (LP-WGS)	STDEV	#QC Sets Assessed	Avg. %Genome Changed REF Material Only (LP-WGS)	STDEV
Synovial sarcoma	2	6	83.70	6.38	5	0.00	0.000	2	0.00	0.000
Ewing sarcoma/Peripheral PNET	2	7	92.34	4.69	5	0.10	0.001	2	0.00	0.000
Carcinosarcoma of the uterus	2	10	82.21	10.52	9	0.15	0.001	6	0.00	0.000
Merkel cell tumor	2	7	88.52	11.30	6	0.32	0.002	3	0.50	0.006
Mesothelioma	2	6	70.68	12.07	5	0.75	0.006	2	0.29	0.002
Ewing sarcoma/Peripheral PNET	2	11	94.72	3.64	10	1.00	0.008	5	0.50	0.005
Merkel cell tumor	2	6	92.84	5.84	5	1.04	0.003	2	1.23	0.011
Mesothelioma	2	6	85.42	6.33	5	1.36	0.005	2	0.98	0.009
Liposarcoma	2	10	49.92	20.38	9	1.39	0.010	5	0.56	0.005
Rhabdomyosarcoma, NOS	2	10	80.49	9.46	9	1.50	0.005	5	1.23	0.010
Hurthle cell neoplasm (thyroid)	2	9	46.79	12.02	8	1.69	0.009	4	1.10	0.010
Osteosarcoma	1.5	8.5	76.43	13.79	7	1.84	0.017	5	0.14	0.002
Neuroendocrine cancer, NOS	2	6	87.02	8.21	5	2.04	0.009	2	2.65	
Adenocarcinoma - small intest.	2.5	9.5	61.54	13.42	8	3.66	0.028	5	2.74	0.017
Osteosarcoma†	2	10	49.68	10.16	13†	4.73	0.012	10†	5.30	0.023
Malig. periph. nerve sheath tum.	2	7	66.77	15.10	6	7.07	0.011	3	6.00	0.017
Rhabdomyosarcoma, NOS	2	6	52.40	13.86	5	8.28	0.001	2	8.21	0.040
Penile SCC (epidermoid)	2	6	57.75	4.47	4	9.54	0.015	2	Pending	
Neuroendocrine cancer, NOS	2	6	84.82	9.11	5	16.07	0.055	2	19.97	0.060
Average			**73.85**			**3.18**			**2.76**	

*Passages determined from initial implant material. If a passage 1 and passage 2 vial were used for initial implantation and then mice were randomized for the drug study, the average passage of the study is 1.5.
†Model restarted from cryomaterial due to human DNA content of <20% in Passage 11 QC material; excluded from analysis above.

Visual Assessment of Response to Fast-Track Single-Agent Follow-up Studies

Qualitative Visual Assessment	
Bin 1	CR Achieved, >1 timepoint (<60mm^3)
Bin 2	Tumor regressed ~30%, durable response (~1 cy)
Bin 3	Tumor regressed ~30% >1 timepoint, regrew at drug removal
Bin 4	Tumor stasis, durable response (~1 cy)
Bin 5	Tumor stasis, regrew at drug removal
Bin 6	Slowed, but Progressive Growth
Bin 7	Grew at Same Rate as Control

✓ Perform Single Agent Studies
✗ No Further Studies at this Time
? Additional Studies Needed



- Tumor volume curves are visually assessed while drug study is on-going to determine trend of response
- Binning of visual assessment is used to categorize response. Combinations that are active in 30%-50% of the models are prioritized for single-agent testing
- Pie charts represent visual response assessment of all models treated with therapeutic combinations, irrespective of histology
- All studies maintained until subcutaneous tumor burden is ≥1000 mm^3 at which point quantitative response assessment will be performed

Summary

- Due to the large study size, a visual assessment of depth and durability of response was used to fast-track drug combinations for single-agent follow-up studies.
- Bookend studies indicate some variability in consistency of response across passages. Further analysis including increased numbers of drug studies with bookends and NGS of tumor material from the studies will be performed to identify any underlying factors.
- Overall genome heterogeneity of models across passages is consistent with the intra-model heterogeneity observed at baseline by low-pass whole genome sequence assessment.
- Single agent studies are beginning to identify drug combinations with increased efficacy over single agents. Combinations where this holds in a larger percentage of all models, or within a specific rare disease type, will be moved forward to full efficacy and biomarker studies.

This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. NCI-Frederick is accredited by AAALACi and follows the Public Health Service Policy on the Care and Use of Laboratory Animals. These studies were conducted on an Institutional Animal Care and Use Committee approved protocol.