

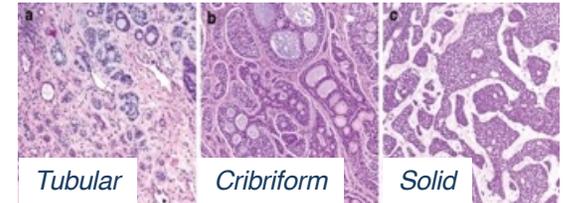
ACC PDX-Derived 3D Cell Culture Platform

Jeff Kaufman, Executive Director, ACCRF

Nikki Spardy Burr, Director of Research, ACCRF



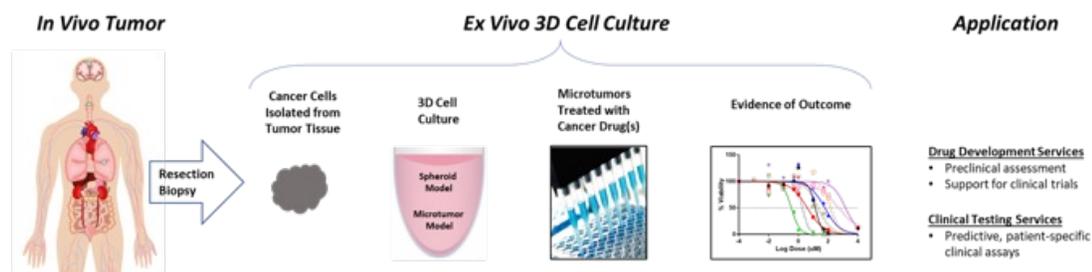
ACC Disease and Model Overview



- ACC is a rare, glandular cancer that typically arises in the salivary glands.
- ACC tumors display various histologies, including cribriform, tubular and/or solid patterns. Cribriform and tubular tumors are comprised of myoepithelial and epithelial cell populations; whereas solid tumors shift to primarily epithelial cells.
- MYB-driven ACC GEMMS have been published but have long tumor latency.
- 20+ PDX models that faithfully recapitulate the disease (histology and genomics) are managed/screened by ACCRF via a CRO (XenoSTART; San Antonio, TX). Cost and time can be an obstacle for testing compounds early in drug development.
- Bona fide 2-dimensional ACC cell lines have been challenging to establish. Mis-identification and loss of important biomarkers (*MYB* fusion- the only recurrent alteration) continue to plague the field. You can read more about 2D ACC cell line development on ACCRF's [webpage](#).
- Goal: Develop valid *in vitro* ACC models for genomic and chemical screening, target validation and drug development.

Developing 3D Cultures From ACC PDX tissue

Kiyatec's ex vivo platform:

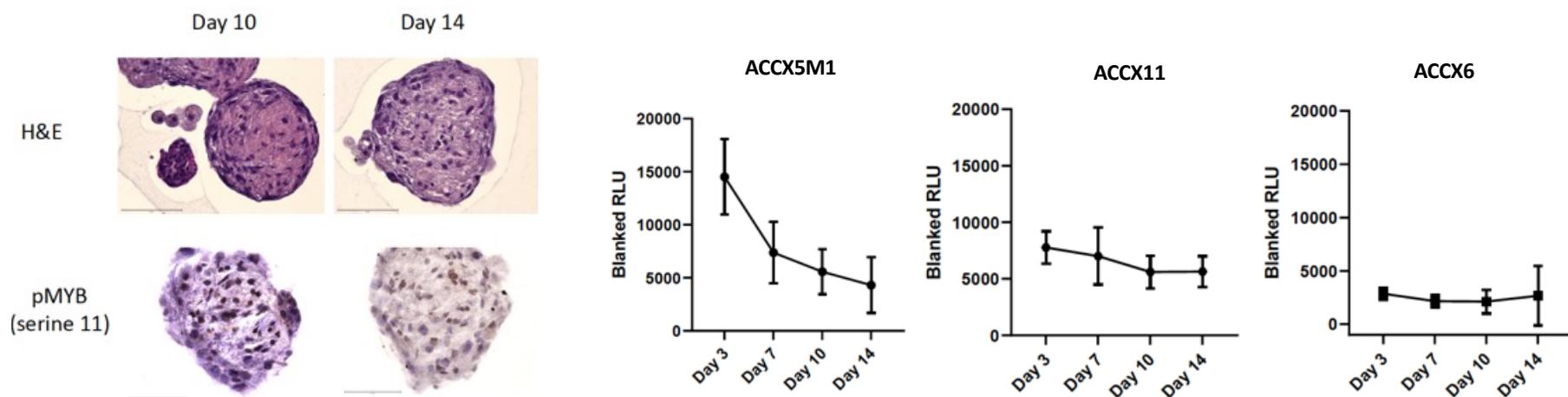


- ACCRF partnered with Kiyatec, a 3D cell culture platform, to develop 3D cultures from ACC PDX tissue.
- The goals of the project were to:
 - Explore growth of cells from 4 different ACC PDX models in Kiyatec's platforms: spheroid (short-term growth) and microtumor (long-term growth),
 - Confirm expression of ACC biomarkers (MYB, NOTCH, p63, KIT and Ki67) over time in culture,
 - And perform a 3D drug screening using novel drugs and compounds previously screened in vivo.

Visit Kiyatec's [website](#) to learn more about their ex vivo platforms and [clinical trial](#) designed to test the predictive power of their ex vivo 3D assay in patients with advanced cancers, including rare diseases.

ACC Spheroids: Example Histology, MYB Staining and Proliferation

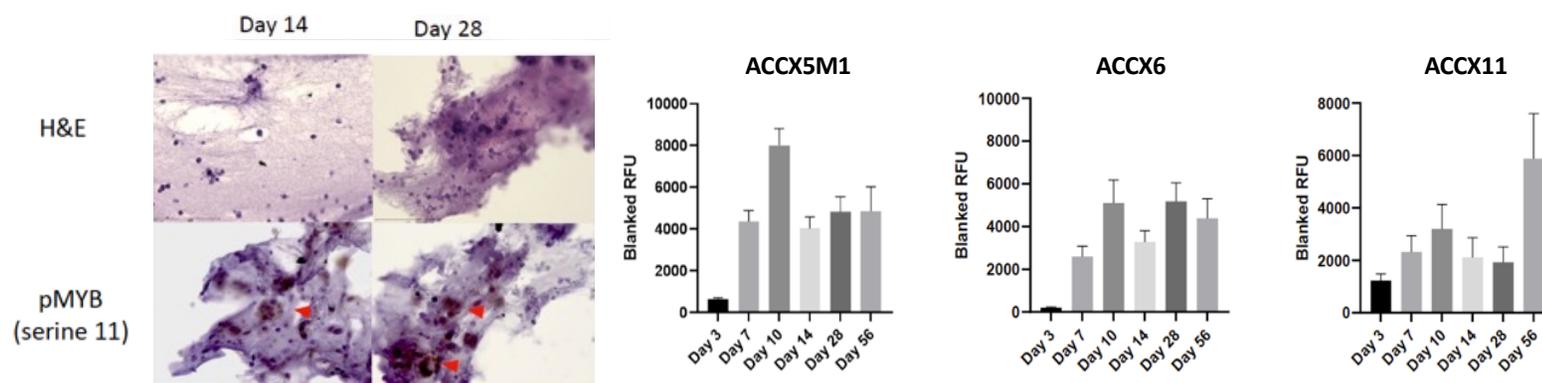
ACC5M1, cribriform histology, MYB+, NOTCH-inactivating mutation PDX model



- ACC5M1, ACCX6 and ACCX11 PDX cells formed tight spheroids up to day 14 with CellTiter-Glo levels suitable for drug screening. ACCX9 cells failed to grow robustly in the spheroid platform (data not shown).
- A reduction or plateau in viability is attributed to model maturation (stabilization of growth:death rate) given healthy morphology and positive Ki67 staining (data not shown).
- PDX-derived spheroid models showed some level of MYB staining during the timeline explored.

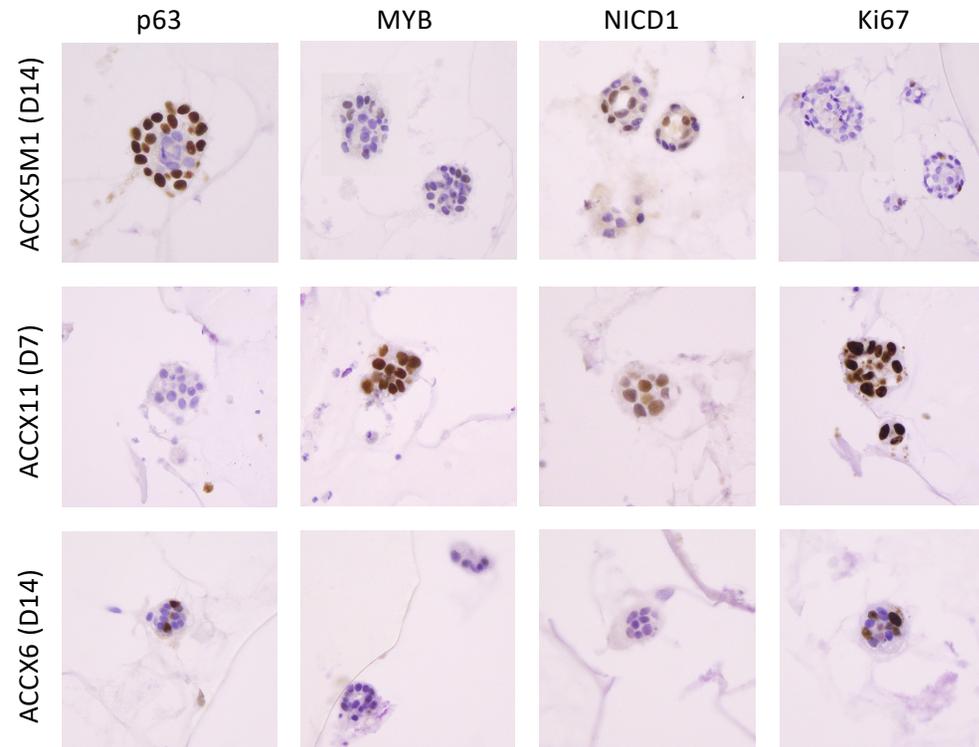
ACC 3D Microtumors: Example Histology, MYB Staining and Proliferation

ACCX11, solid histology, MYB+, NOTCH1-mutant PDX model



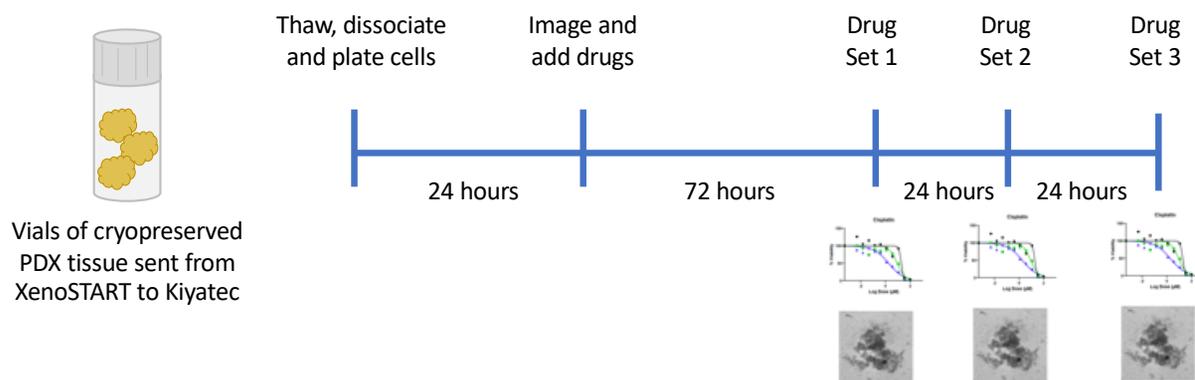
- Microtumors formed readily from ACC5M1, ACCX6 and ACCX11 PDX cells; less so from ACCX9 (not shown).
- A reduction or plateau in viability (measured by PrestoBlue) over time was again attributed to model maturation given healthy morphology of structures and positive Ki67 staining (see slide 6).
- Tissue maturation was detected at later time points. Red arrows in the above image point to acinar-like structures
- MYB staining was observed in ACC5M1, ACCX6 and ACCX11 microtumors up to day 28.

Extensive ACC Biomarker Validation (performed by Dr. Jon Aster, BWH Path)



- ACCX5M1, ACCX6 and ACCX11 microtumors stain positive for p63 and NOTCH in expected histological patterns.
- MYB staining is highest in ACCX11, with weaker positivity observed in ACCX5M1 and ACCX6.
- Ki67 staining is highest in ACCX11 and ACCX6, with weaker subset positive cells observed in ACCX5M1.

Spheroid Drug Screening Platform



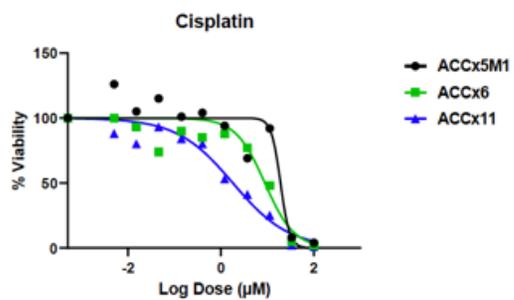
- ACCX5M1, ACCX6 and ACCX11 spheroid models were chosen for the pilot drug screen.
- Screens involve 7 replicates at 10 drug concentrations (typical range, 0.005uM-100uM); run in 384 well plates. Vehicle, DMSO and blanked wells used as controls.
- Optimal assay timepoint determined upfront for drugs not yet screened at Kiyatec.

Drug List And IC50 Table

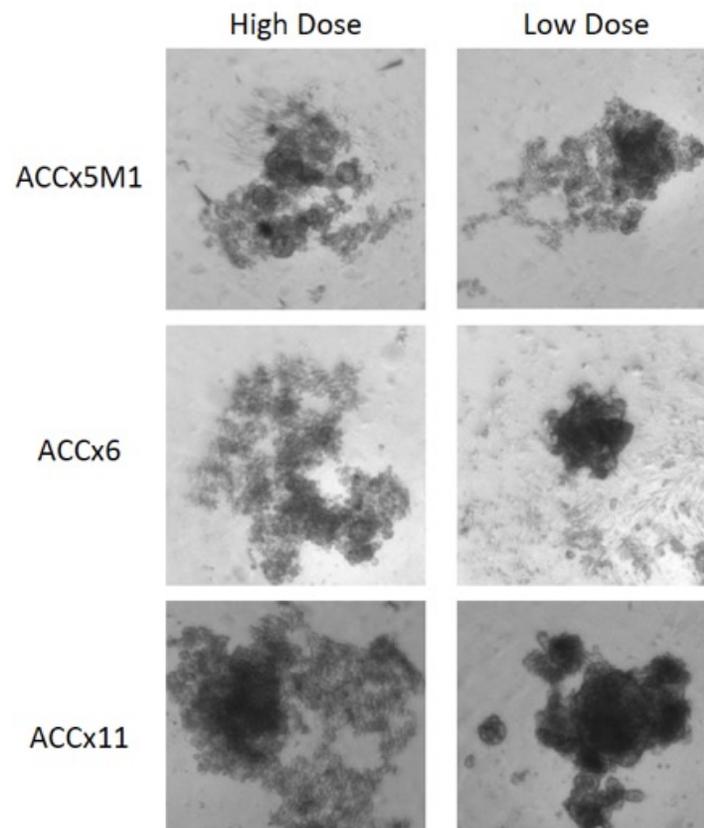
	Drug	Target	Time Point (h)	IC50 (uM)		
				ACCx5M1	ACCx6	ACCx11
1	Cisplatin	Chemo- DNA Damage	72	19.16	8.808	1.803
2	Paclitaxel	Chemo- Microtubule Poison	72	No Response	No Response	0.2119
3	Etoposide	Chemo- DNA Damage	72	40.01	11.1	1.102
4	Docetaxel	Chemo- Microtubule Poison	72	No Response	No Response	0.4712
5	Abemaciclib	CDK4/6	72	2.565	1.672	1.222
6	Olaparib	PARP	72	62.09	3.817	8.409
7	Lapatinib	EGFR	72	5.64	2.514	4.021
9	Tazemetostat	EZH2	72	No Response	No Response	5.943
10	Vinorelbine	Chemo- Microtubule Poison	72	No Response	No Response	0.008922
11	CB-103	NOTCH	72	No Response	No Response	No Response
13	APG-115	MDM2	72	6.186	12.37	4.305
15	BMS906024 (AL101)	NOTCH	72	No Response	No Response	No Response
21	Bortezomib	Proteasome	96	0.0309	0.0114	0.0043
22	Cabozantinib	VEGF, MET	96	22.21	15.12	7.734
23	Everolimus	MTOR	96	80.45	22.13	0.2048
24	Gemcitabine	Chemo- DNA Synthesis	96	No Response	No Response	2.702
25	Imatinib	BCR-ABL, KIT	96	21.88	14.76	6.528
26	Vorinostat	HDAC	96	5.196	5.153	3.86
27	Levatinib	VEGF	96	95.21	17.88	5.654
28	Sorafenib	VEGF	96	29.56	25.33	13.54
29	Apatinib	VEGF	96	No Response	No Response	2.756
30	Axitinib	VEGF	96	No Response	No Response	2.2
31	AZD4573	CDK9	96	0.0024	<OOR	0.0002186
38	ATRA	RA, MYB	120	No Response	No Response	No Response
44	GSK3326595	PRMT5	120	87.72	25.29	No Response

Drugs screened across PDX models

Example Results- Cisplatin

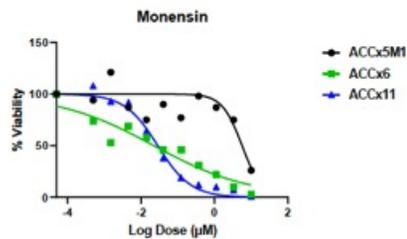


PDX	IC50 (µM)
ACCx5M1	19.16
ACCx6	8.808
ACCx11	1.803



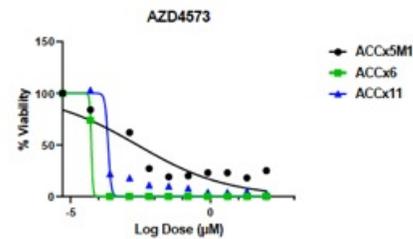
3D Models Show Sensitivity To Drugs With Link To MYB Inhibition

Monensin



PDX	Monensin IC_{50} (μM)
ACCx5M1	5.661
ACCx6	0.02252
ACCx11	0.02868

AZD4573

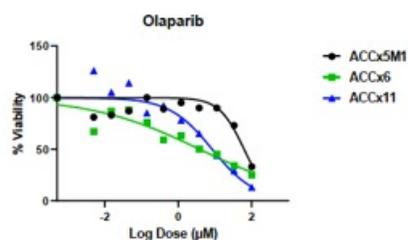


PDX	AZD4573 IC_{50} (μM)
ACCx5M1	0.0024
ACCx6	0.00005491
ACCx11	0.0002186

- Monensin (identified as a MYB inhibitor in MYB reporter screens) shows anti-proliferative activity in leukemia and primary ACC cells ([Yusenko et al., Cancer Lett. 2020](#)).
- Other CDK9 inhibitors shown to inhibit MYB expression in ER+ breast cancer cells via intron pausing ([Mitra et al., Nucleic Acids Res. 2012](#) and [Mitra et al., Oncotarget, 2016](#)).

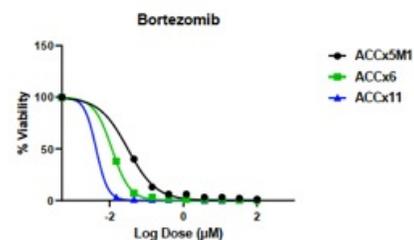
3D Models Reveal Unexpected Sensitivity To Certain Drugs

Olaparib



PDX	Olaparib IC ₅₀ (µM)
ACCx5M1	62.09
ACCx6	3.817
ACCx11	8.409

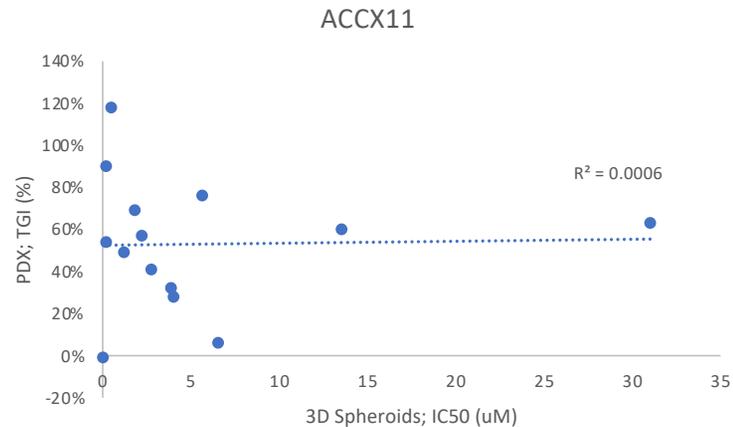
Bortezomib



PDX	Bortezomib IC ₅₀ (µM)
ACCx5M1	0.03086
ACCx6	0.01143
ACCx11	0.004275

- IC50s for Olaparib in ACCX6 and ACCX11 spheroids are on par with activity seen in breast/ovarian BRCA-mutant models at Kiyatec.
- Sensitivity of ACC PDX 3D spheroids to Bortezomib is on par with AML cell lines.

Comparing Baseline Drug Activity Across 3D Vs. PDX ACC Models



- ACC 3D assays do not appear generally predictive of PDX model sensitivity due to a variety of reasons:
 - Anti-angiogenesis factors (like TKIs) are unlikely to work in 3D systems lacking blood vessels.
 - Some drugs (like ATRA) appear to broadly lack activity across Kiyatec's 3D models via an unknown mechanism.
 - Alternative assay readouts (cell cycling, apoptosis, differentiation, etc.) may be more informative.
- Until relative IC50 values can be assessed across a larger panel of ACC lines, 3D models may be most useful to assess short-term drug-target engagement and whether drug combinations or analogs shift IC50 values.

ACCRF 3D Summary

- Spheroid and microtumor models with suitable growth kinetics and proper biomarker expression were established from 3 out of 4 ACC PDX models tested (ACCX5M1, ACCX6 and ACCX11).
- Evidence of cell maturation in microtumors may allow exploration of more complex mechanisms like differentiation, etc.
- IC50 drug curves generated across 3 ACC PDX spheroid models reveal:
 - Sensitivity to drugs that have been linked to MYB inhibition
 - Sensitivity to drugs with no obvious link to ACC biology
 - 3D sensitivity to drugs is not globally predictive of PDX activity
- Kiyatec has also successfully generated PDX-derived organoid lines. Molecular characterization of these models is currently underway by ACCRF grantees.
- In 2024, Kiyatec discontinued all work on ACC 3D cultures. ACCRF is currently exploring new CROs to partner with on the development of new, 3D ACC models.