Adenoid Cystic Carcinoma Research Foundation

THE LANDSCAPE OF ACC RESEARCH: PAST, ONGOING AND FUTURE EXPLORATIONS

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Introduction

In October 2006, ACCRF drafted its first *Research Agenda* to accelerate the development of improved therapies and a cure for ACC. The ACCRF Scientific Advisory Board outlined a program of directed research to address three key issues:

- **Resources** – Biospecimens, cell cultures and animal models as the building blocks for research
- **Mechanisms** – Identification and validation of therapeutic targets
- **Translation** – Preclinical studies to facilitate promising clinical trials

In the past seven years, ACCRF's network of affiliated researchers has produced an admirable record of achievement (Appendix A). The components of the *Research Agenda Framework* (Appendix B) have remained largely stable over that period as have the foundation's *Guiding Principles* (Appendix C). Given the successful accomplishment of many initial objectives, the Scientific Advisors recently decided that it would be useful to undertake a strategic discussion of future research priorities for the field and the foundation.

This working document seeks to describe (i) findings from recent studies, (ii) objectives of ongoing studies and (iii) key questions that remain unexplored. Ideally, this document will benefit from the constructive and continuing feedback of interested parties and will serve as a useful map for researchers seeking fruitful avenues to pursue in the coming 3-5 years.

Past Explorations and Findings

A. Resources: Biobanking – ACCRF grants to MD Anderson and the University of Virginia invigorated biospecimen banking efforts, eventually culminating in the funding by the National Institute of Dental and Craniofacial Research (NIDCR) of the Salivary Gland Tumor Biorepository. Centralized at MD Anderson, the collaboration among several leading institutions continues to add to its collection of hundreds of high-quality frozen specimens that are available to researchers globally.

B. Resources: Cell Lines – Six cell lines that were purported to be derived from ACC were found to be cross-contaminated and misidentified. Other non-immortalized cell lines have not been validated. Two ACC cell lines immortalized with HPV have been widely distributed though they no longer may

have the t(6;9) translocation of the associated primary tumor and early passages. Several labs have attempted to develop new validated and non-immortalized cell lines, though none have been successful yet.

C. **Resources: Patient-Derived Xenograft (PDX) Mouse Models** – The University of Virginia has developed approximately 20 PDX models that retain the histology, chromosomal translocations and gene expression of the associated primary ACC tumors. The models have been published and extensive genomic and drug screening data is available on a web portal. Seven of the PDX models have been transferred to South Texas Accelerated Research Therapeutics (START) where ACCRF maintains them on a platform open to all researchers for drug screening studies.

D. **Mechanisms: Chromosomal Rearrangements** – Five key studies have been published on ACC translocations. A reasonable estimate is that 55% of cases have the "canonical" MYB-NFIB fusion gene, another 15% involve a fusion of MYB with another gene, and another 15% involve a non-MYB gene (NFIB, AIG1, etc.) on chromosomes 6 and/or 9. Myb (or at least its first few exons) is over-expressed in 80-95% of cases.

- Recurrent fusion of MYB and NFIB transcription factor genes in carcinomas of the breast and head and neck (September 2009).
- Comprehensive Analysis of the MYB-NFIB Gene Fusion in Salivary Adenoid Cystic Carcinoma: Incidence, Variability and Clinicopathological Significance (August 2010).
- MYB Expression and Translocation in Adenoid Cystic Carcinomas and Other Salivary Gland Tumors With Clinicopathologic Correlation (January 2011).
- Analysis of MYB expression and MYB-NFIB gene fusions in adenoid cystic carcinoma and other salivary neoplasms (September 2011).
- Novel Chromosomal Rearrangements and Break Points at the t(6;9) in Salivary Adenoid Cystic Carcinoma: Association with MYB–NFIB Chimeric Fusion, MYB Expression, and Clinical Outcome (October 2011)

E. **Mechanisms: Genomics** – Two key studies have been published on next-generation sequencing of ACC, covering 79 exomes and 5 whole genomes. The major themes from the Memorial Sloan-Kettering team of researchers were remarkably consistent with those of the MD Anderson/Sanger Institute team. First, the ACC genome is relatively quiet compared to other solid tumors, with a mean of about 12 nonsynonymous somatic mutations per case. Second, the prevalence of MYB-NFIB fusions tracks closely with the aggregated findings of the five articles above, solidifying MYB as the most frequent genetic alteration across ACC. Third, the only recurrent copy number variations involved deletions of 1p, 6q, 9p, 12q and 14q. Fourth, chromatin regulators were mutated in between one-third and one-half of cases. Fifth, known cancer genes were mutated in

small but significant subsets, particularly in the FGF, IGF, PI3K and NOTCH pathways. And, finally, the MD Anderson/Sanger team identified SPEN as a novel cancer gene that may be involved in NOTCH signaling.

- The mutational landscape of adenoid cystic carcinoma (May 2013)
- Whole exome sequencing of adenoid cystic carcinoma (June 2013)
- Mutation signature of adenoid cystic carcinoma: evidence for transcriptional and epigenetic reprogramming (Commentary - June 2013)

F. **Mechanisms: Methylation** – Methylation profiling has been conducted by Johns Hopkins and MD Anderson. AQP1 was identified as an oncogene candidate in ACC. Promoter hypomethylation does not appear to explain the differential expression of MYB in ACC.

G. **Mechanisms: Phosphoproteomics** – A collaboration between Cell Signaling Technology and the University of Virginia studied the phosphorylation of tyrosine kinases in ACC primary and xenograft tumors. Amongst others targets, FGFR, PDGFR and IGF-1R were identified as activated in most cases.

H. **Translation: Preclinical Drug Screening** – In the absence of appropriate ACC cell lines, the focus of preclinical drug screening has been on the PDX models. Approximately 35 approved anticancer agents and 35 novel agents have been studied. Beyond baseline studies to determine the sensitivity of each PDX model to chemotherapy, anticancer agents are selected for screening based on whether they inhibit a target implicated in ACC. Additionally, agents being studied in clinical trials of ACC patients are screened in the PDX models to correlate activity in humans and mice. Results for one novel agent, Dovitinib, were sufficiently promising to move forward to clinical trials the concept of FGFR inhibition in ACC.

I. **Translation: Clinical Trials** – The primary goal of ACCRF is to help develop science-driven concepts leading to clinical trials of treatments that alter significantly the trajectory of the disease across the ACC patient population. Unfortunately, the history of ACC clinical trials indicates that no systemic therapies have resulted in substantial and sustained tumor regressions in a significant subset of patients. Until very recently, ACC patients have been accrued to small studies without any clear mechanistic rationale or preclinical drug screening data to suggest activity of the agents in ACC. However, the quality of clinical trials is improving and some recent publications and abstracts have pointed to targeted therapies (Dovitinib, Sorafenib, Sunitinib and Vorinostat) with extended periods of stable disease and a few objective responses in progressing ACC patients. In September

2013, the first ever orphan drug designation for ACC was granted to Dovitinib by the U.S. Food and Drug Administration.

Ongoing Investigations

J. **Resources/Mechanisms: Genetically-Engineered Mouse Models (GEMMs)** – Various approaches to developing GEMMs are being attempted at Dana-Farber, the University of Virginia, MD Anderson and the University of Queensland. Different promoters, MYB-NFIB fusion variants and crosses are being explored. Preliminary findings suggest one of the attempts may have a phenotype with high penetrance. Once developed, this portfolio of diverse models will be crucial for functional studies and drug screening.

K. **Mechanisms: Genomics** – Multiple efforts are under way to sequence additional exomes and whole genomes of ACC specimens. The Sanger Institute, MD Anderson and Memorial Sloan-Kettering are working on expanded cohorts of cases, while Johns Hopkins and TGEN also will be completing studies in the coming year. In total, approximately 200 exomes will be completed along with perhaps 35 whole genomes. In addition, copy number variations and gene expression studies will be carried out in parallel. A subset of cases also will be analyzed on more advanced transcriptome sequencing and methylation binding domain bisulfite sequencing platforms. Initial discussions are under way to create a web portal that will make the various data sets accessible to qualified researchers.

L. **Mechanisms: Validation of Myb as a Therapeutic Target** – Industry sponsors will invest in drug development and/or clinical trials for only targets that are well validated. While the high incidence of MYB rearrangements and myb over-expression in ACC makes it very likely – perhaps 80% – that myb is a therapeutic target, further validation is needed. It must be shown not only that myb over-expression contributes to tumorigenesis, but also that it is necessary for the maintenance of ACC tumors. The University of Virginia is using a lentivirus mediated shRNA knockdown approach to test the significance of the fusion protein in maintaining the transformed phenotype of ACC xenografts.

M. **Mechanisms: Downstream and Upstream Targets of Myb** – Until myb becomes part of the druggable genome and may be targeted directly, an opportunistic strategy of targeting myb's downstream and upstream targets is appropriate. Massachusetts General Hospital (MGH) is performing genome-wide ChIP-seq for 5 histone marks to identify active genes, promoters, enhancers, bivalent genes, inactive genes and heterochromatin in primary ACC tumors. The team also is performing myb ChIP-seq studies to identify which gene promoters are bound by myb in ACC

tumors. Comparisons of the two data sets along with gene expression data will help narrow down potentially active therapeutic targets, including chromatin modifiers necessary for maintaining the specific chromatin landscape in the presence of myb. Prior myb ChIP-seq studies were carried out in other tumor types at the University of New Mexico (breast cancer cells; T-cell leukemia throughout the cell cycle) and the University of Queensland (myb-transformed myeloid cells).

N. **Mechanisms: Activities of MYB fusion variants** The recurrent t(6;9) translocation is promiscuous. The MYB-NFIB fusion gene has a wide range of breakpoints spanning exons 7 through 15 of MYB. The University of New Mexico is studying the differences in the transcriptional activity of wild-type MYB and MYB-NFIB fusion variants using DNA binding and transcriptional activation assays. In addition, the researchers are comparing whether the same target gene promoters are bound by the variants.

O. **Translation: High-Throughput Drug Screening in Myb-GFP Zebrafish** – At Harvard, a screen of 2,500 compounds of known action in zebrafish embryo cell cultures has identified many that suppress and increase myb. This subset of compounds was then screened in whole zebrafish embryos to identify potential drugs and to indicate pathways to explore further with more specialized libraries. Promising drugs will be screened in the PDX models.

P. **Translation: Preclinical Drug Screening and Clinical Trial Concepts** – Based on the findings of genomic studies of ACC, several pathways appear to be implicated in the progression of the disease. Accordingly, ACCRF has accessed inhibitors of the FGF, IGF and NOTCH pathways as well as chromatin remodeling for tumor growth inhibition studies in the PDX models. Some studies also include pharmacodynamic endpoints. Promising data is shared, subject to confidentiality provisions, with potential sponsors of clinical trials.

Potential Future Explorations

Combining the findings on MYB rearrangements and somatic mutations, a vague picture of ACC comes into focus. On the one hand, ACC appears to be a homogeneous disease in that MYB is implicated in the preponderance of cases. However, beyond MYB, ACC becomes a very heterogeneous disease with many low-prevalence genetic alterations, perhaps explaining the variable course across patients. A reasonable hypothesis is that MYB is necessary – and probably sufficient – for indolent disease, while additional genetic hits account for aggressive cases.

Given this context, ACCRF's Scientific Advisory Board recently met to discuss potentially fruitful avenues of research. What follows are some key themes and related questions for the ACC research community to consider. Some questions will be answered partially by ongoing projects. Some may not be answerable for many years. And some may turn out to be unimportant. The list is merely a starting point for discussion and will improve as feedback is gathered and incorporated. Almost certainly, difficult questions will call for multiple, complementary approaches to finding answers.

1. **Resources: Data Integration** – Within the next year, past and ongoing projects will have generated enormous, varied and robust data sets from well-annotated biospecimens. The curation and integration of these data sets into a centralized and open bioinformatics platform would maximize the value of these resources. ACCRF will focus on working with the relevant parties to establish the data needs, inter-operability, governance, linkage platforms and access policies that will permit researchers to generate promising hypotheses.

2. **Resources: Cell Line Development** – Validated cell lines would accelerate many aspects of ACC research through (i) functional studies of molecular pathways, (ii) RNA interference studies to identify therapeutic targets, and (iii) high-throughput drug screening to identify active compounds. How might the lack of these vital resources be addressed through more grants, a formal consortium or prizes?

3. **Resources: Patient Derived Xenograft (PDX) Models** – Analysis of the forthcoming genomic data sets may lead to more clearly defined clusters/subsets of ACC patients whose tumors are driven by particular combinations of molecular drivers. ACCRF should support the development of several PDX models for each cluster/subset of ACC in order to recapitulate for preclinical studies the genomic diversity of the disease. Each PDX model should be extensively characterized to appraise its molecular alterations.

4. **Mechanisms: Tumor Heterogeneity** – Aside from molecular differences between patients, there is growing awareness of molecular differences within each patient's tumors at any point in time as well as over time. A single ACC tumor may have components of all three histologic subtypes (cribriform, tubular and solid), perhaps reflective of underlying molecular differences. And some metastases harbor alterations that result from clonal evolution, perhaps leading to variable responses to targeted agents of different tumors within the same patient. How may this intra-patient tumor heterogeneity be analyzed to understand the varying aggressiveness and treatment responses of the disease?

5. **Mechanisms: MYB** – Given the high prevalence of rearrangements and over-expression of MYB in ACC, it is crucial to delve into its significance.
   - Is MYB a validated target in ACC? Or are particular fusion variants the drivers of progression?
   - What are the essential downstream targets of MYB or its fusion variants?
   - Several MYB cofactors have been identified in non-ACC tumor types: P300, Menin, FLASH, mi-2α, HIPK1. Are there more cofactors and which are necessary for ACC progression? Are they valid targets?
   - Are there viable upstream targets of MYB to exploit?
   - Assuming MYB is a validated target, what approaches might directly interfere with its activity? Small molecules, interference with cofactors, transcriptional elongation, stapled peptides, antisense and RNAi are some approaches to explore.
   - How good a target is MYB from a toxicity perspective? Might mouse models elucidate the effect of MYB inhibition on normal cells?

6. **Mechanisms: Epigenetics** – The initial data on somatic mutations suggest an important role for chromatin remodeling factors in ACC. Although preliminary methylation studies have not identified any potential targets, a more comprehensive epigenetics effort should be undertaken. Initial **phosphoproteomics** studies have been fruitful and they should be expanded.

7. **Mechanisms: Cancer Stem Cells** – The cancer stem cell hypothesis appears to fit the data for only some tumor types. A recent review describes some preliminary support for the hypothesis in ACC, but more explorations are warranted.

8. **Mechanisms: Immunotherapy** – Recent successes in melanoma and leukemia merit an appraisal of whether ACC might be treated with an immunotherapy approach. How might we assess its likelihood of success in ACC? Few lymphocytes are found in ACC tumors, but it is not clear why.

9. **Translation: Preclinical Screening of Drug Combinations** – Single agent regimens generally have not been highly active in ACC patients or preclinical models. Among drugged pathways, there are only a few that appear to be involved in ACC progression (FGFR, IGF-1R, NOTCH, HDAC and PI3K). The potential drug combinations are sufficiently manageable as to be attempted in the PDX models. Such studies should be pursued.

10. **Translation: Patient Registry** – Biorepositories have been incredibly helpful in supporting studies to identify genetic alterations and molecular targets. However, they often lack the clinical outcomes data that will be necessary for (i) natural history studies, (ii) identification of prognostic and

predictive markers, and (iii) long-term analyses of treatment efficacy. A centralized registry with clinical outcomes and tumor profiling data would facilitate these types of studies while permitting patient stratification for precision medicine and targeted clinical trials. How many patients would be needed over what horizon to answer which specific research questions? Might existing biorepository or registry efforts be expanded to accomplish the goals?

11. **Translation: Clinical Trials** – ACCRF must continue to facilitate the initiation of promising clinical studies based on mechanistic understanding of ACC as well as preclinical drug screening data. However, this effort may be complicated if there are proliferating clusters/subsets of ACC tumor profiles that respond differently to treatments. Must all patients have their tumors profiled? Should they be assigned to ACC-specific studies or placed in broader cohorts defined by genetic alterations? What are the regulatory implications for drug approvals in this environment? These issues are not specific to ACC, but must be addressed, perhaps with a patient registry.

Conclusion

Over the past several years, the ACC research community has begun to paint an intelligible landscape of the disease. The specimens and models were the canvas, brushes and paints. And the genomic studies sketched out the forests. Now it is time to fill in the details of the trees.

From the ongoing projects, investigators very soon will have a clear catalogue of what is altered in ACC. The emphasis of research will shift more towards functional studies that explain how those alterations interact with each other and how their negative impacts may be mitigated or eliminated in patients.

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APPENDIX A: ACC RESEARCH ACCOMPLISHMENTS

	2005	2013
Biobanking	Limited	Repositories with hundreds of frozen tumor specimens
Cell Lines	Multiple invalid models	Misidentifications discovered; valid models in development
Animal Models	None	17 mouse xenografts developed; progress in transgenic models
Genomics	Sporadic reports of translocations	• Discovery of recurrent t(6;9) and MYB-NFIB fusion gene • Exome and whole genome sequencing, CGH, methylation, gene expression and phosphorylation studies completed • Investigation of downstream targets
Preclinical Drug Screens	None in valid models	• Open xenograft platform for academia and industry • Strong relationships with biopharmaceutical companies • 35 approved and 35 novel compounds screened in xenografts
Mobilizing Patients	Limited	Tissue donations, clinical trial accrual and $9 million in donations
NIH Commitments	Negligible	Over $20MM for salivary gland tumor research (NIDCR)
Clinical Trials	Few & Opportunistic	Multiple science-driven studies with correlative studies, supported by preclinical drug screening

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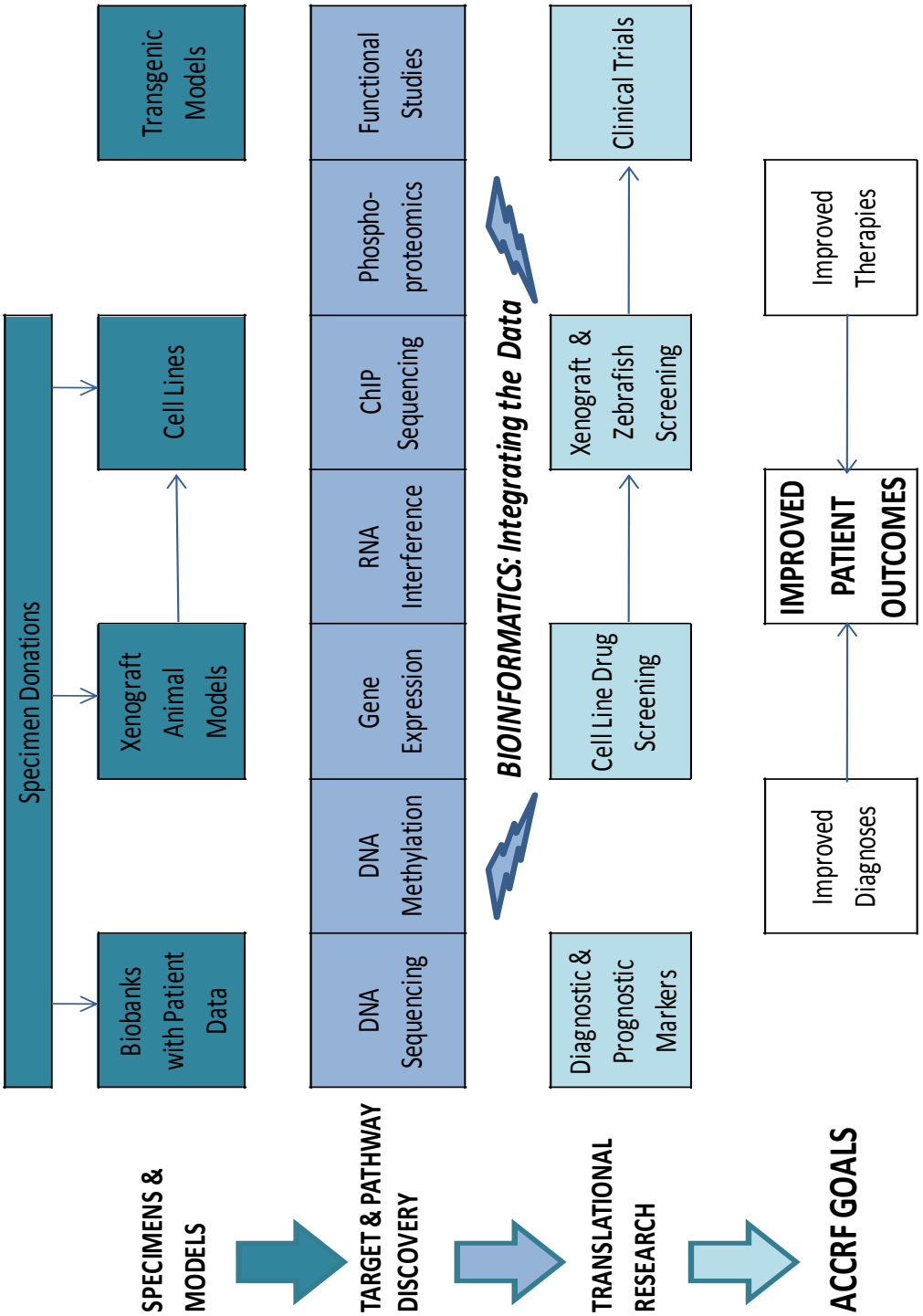
APPENDIX B: ACCRF RESEARCH AGENDA FRAMEWORK



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APPENDIX C: ACCRF GUIDING PRINCIPLES

Venture Philanthropy – Development of translational concepts leading to clinical investigations

Proactive Project Selection – Directed research guided by the Scientific Advisory Board

Portfolio Management Perspective – Diversity of approaches without all eggs in one basket

Multi-Institutional Cooperation – Division of labor to the most appropriate investigators

Accelerating Practices – Clear deliverables with expectations of sharing across the network

Disease-Specific – Focus on the peculiarities of ACC while incorporating general approaches