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BANBURY WHITE PAPER

Rhabdomyosarcoma: Current Challenges and Their Implications for Developing Therapies

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Rhabdomyosarcoma (RMS) represents a rare, heterogeneous group of mesodermal malignancies with skeletal muscle differentiation. One major subgroup of RMS tumors (so-called "fusion-positive" tumors) carries exclusive chromosomal translocations that join the DNA-binding domain of the *PAX3* or *PAX7* gene to the transactivation domain of the *FOXO1* (previously known as *FKHR*) gene. Fusion-negative RMS represents a heterogeneous spectrum of tumors with frequent *RAS* pathway activation. Overtly metastatic disease at diagnosis is more frequently found in individuals with fusion-positive than in those with fusion-negative tumors. RMS is the most common pediatric soft-tissue sarcoma, and approximately 60% of all children and adolescents diagnosed with RMS are cured by currently available multimodal therapies. However, a curative outcome is achieved in <30% of high-risk individuals with RMS, including all those diagnosed as adults, those diagnosed with fusion-positive tumors during childhood (including metastatic and nonmetastatic tumors), and those diagnosed with metastatic disease during childhood (including fusion-positive and fusion-negative tumors). This white paper outlines current challenges in RMS research and their implications for developing more effective therapies. Urgent clinical problems include local control, systemic disease, need for improved risk stratification, and characterization of differences in disease course in children and adults. Biological challenges include definition of the cellular functions of PAX-FOXO1 fusion proteins, clarification of disease heterogeneity, elucidation of the cellular origins of RMS, delineation of the tumor microenvironment, and identification of means for rational selection and testing of new combination therapies. To streamline future therapeutic developments, it will be critical to improve access to fresh tumor tissue for research purposes, consider alternative trial designs to optimize early clinical testing of candidate drugs, coalesce advocacy efforts to garner public and industry support, and facilitate collaborative efforts between academia and industry.

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Rhabdomyosarcomas (RMSs) are exceedingly rare and varied mesodermal cancers, linked by their common propensity to undergo aberrant, partial skeletal muscle differentiation (Parham 2001). RMS tumors can occur at any age, but most diagnoses are made in children and adolescents with an annual RMS incidence of 4.3 cases per one million people younger than 20 years of age (Sultan et al. 2009; Perez et al. 2011). There are approximately 350 new diagnoses of childhood RMS in the United States per year. The annual RMS incidence in adults is not entirely clear, but data from the public access Surveillance, Epidemiology and End Results database (Sultan et al. 2009) show that 40% of all RMS tumors are diagnosed in adults >20 years of age.

RMS tumors are typically referred to as muscle cancers because they resemble histologically aberrant muscle differentiation states and often originate in or near muscle beds. Yet, these tumors arise virtually anywhere in the body, including anatomic sites that lack skeletal muscle, such as the biliary and genitourinary tract (Dagher and Helman 1999). Also, cells of both myogenic and nonmyogenic lineage have been shown to contribute to the development of specific RMS-like tumors in mice (Rubin et al. 2011; Hatley et al. 2012). To a great extent, it is unknown to what degree the tissue environment at the primary site, the differentiation state of the cells in which tumorigenic events occur, the specific oncogenic events acquired during transformation, or combinations of these factors determine tumor phenotypes across the RMS spectrum (Hettmer and Wagers 2010).

Differing clinicopathologic RMS phenotypes were recognized first based on their histological appearance and, more recently, based on the genetic makeup of tumors (Parham 2001). The two main histological subtypes diagnosed in the pediatric population are alveolar and embryonal RMS, whereas tumors with pleomorphic and not otherwise specified (NOS) histology account for the majority of RMS diagnosed in individuals >18 years of age (Parham 2001; Sultan et al. 2009; Hawkins et al. 2013). Chromosomal translocations resulting in fusion of the DNA-binding domain of the *PAX3* or *PAX7* genes to the transactivation domain of the *FOXO1* gene (previously known as *FKHR*) have been detected in approximately 55% (*PAX3-FOXO1*) and 20% (*PAX7-FOXO1*) of alveolar histology RMS (Sorensen et al. 2002). Conversely, a small subset of tumors with alveolar histology is fusion-negative (~20%), and nonalveolar (mostly embryonal) RMS tumors never carry *PAX-FOXO1* fusions (Sorensen et al. 2002). Fusion-negative alveolar tumors are clinically and molecularly indistinguishable from the larger group of fusion-negative nonalveolar tumors (Williamson et al. 2010). In contrast, there are marked differences in the genetic makeup of fusion-positive and fusion-negative tumors; for example, fusion-positive RMS tumors have frequent amplification events,

BOX 1. THE BANBURY CENTER AT COLD SPRING HARBOR LABORATORY

The Banbury Center was established in 1977 as a venue for "think-tank" meetings on important topics in molecular biology, genetics, neuroscience, cancer research, and science policy. The center has now hosted more than 600 Banbury Meetings and more than 12,000 participants. Each meeting convenes 20–30 of the leading figures in a field to present and discuss unpublished work, with the aim of identifying the key questions and challenges facing researchers. The Banbury White Papers published in *CSH Perspectives* were devised as a framework for distilling these conclusions and disseminating them to the wider research community.

distinct DNA methylation patterns, and extremely low sequence variation rates, as opposed to loss of heterozygosity at the 11p15.5 locus, frequent chromosomal gains, and a prominent role for RAS pathway activation in fusion-negative tumors (Xia et al. 2002; Langenau et al. 2007; Hettmer et al. 2011; Chen et al. 2013; Schneider et al. 2014; Shern et al. 2014).

Approximately 46% of fusion-positive RMS and 17% of fusion-negative RMS diagnosed in the pediatric age group have formed radiographically detectable metastases at the time of diagnosis, most frequently localizing to the lungs (Williamson et al. 2010; Hawkins et al. 2013). However, even those without detectable metastases almost always carry microscopic seeds of tumor cells in regional lymph nodes and/or distant organs. Effective anti-RMS treatments therefore need to provide control of both the primary tumor (local control) and of distant tumor cell seeds (systemic therapy).

Since the 1970s, collaborative pediatric trials have developed and established multimodal anti-RMS therapeutic strategies, including surgery, radiation, and combinations of conventional chemotherapeutic drugs (Pappo et al. 1995). These therapies have revolutionized pediatric RMS care and achieved 61% overall survival of children and adolescents diagnosed with RMS (Sultan et al. 2009) compared with 25%–30% survival in the 1960s using primarily local therapy (i.e., surgery and radiation) (Pappo et al. 1995). Yet, unfortunately, cure rates have stagnated since the 1990s and distribute unequally across the varied spectrum of this cancer. Of those children diagnosed with *PAX-FOXO1*-positive (including metastatic and nonmetastatic tumors [Williamson et al. 2010; Missiaglia et al. 2012]) or overtly metastatic RMS (including fusion-positive and fusion-negative tumors [Oberlin et al. 2008]), >70% die from their cancer even with the most advanced multimodal therapies. Similarly, >70% of adults diagnosed with RMS succumb to their disease (Sultan et al. 2009). Those who do survive childhood RMS face a lifetime of significant treatment-related effects including profound functional and cosmetic deficits, organ toxicities, and second cancers (Punyko et al. 2005).

Hope for future anti-RMS therapies may rest in large part on interventions that recruit antitumor immune mechanisms, induce myogenic differentiation, or interfere with specific cellular and molecular mechanisms that drive the malignant behavior of RMS cells (Ciarapica et al. 2013; Highfill et al. 2014; Walters et al. 2014). Unfortunately, the preclinical anti-RMS activity of targeted agents such as mTOR inhibitors (Hosoi et al. 1999) and neutralizing IGF1 receptor antibodies (Mayeenuddin et al. 2010) have not translated thus far into durable objective response rates in early-phase clinical trials (Geoerger et al. 2012; Pappo et al. 2014; Weigel et al. 2014). However, improved event-free survival of individuals with relapsed RMS after treatment with the mTOR inhibitor temsirolimus in combination with vinorelbine and cyclophosphamide supports experimental strategies involving combinations of targeted agents and established drugs (Mascarenhas et al. 2014). In May 2014, a group of skeletal muscle and sarcoma biologists, clinicians, and patient advocates met for 3 days at the Banbury Center at Cold Spring Harbor, NY (Box 1) to discuss current challenges in RMS research and opportunities for developing therapies with the long-term goal of making RMS a uniformly survivable disease. This white paper outlines the clinical and biological problems (identified by this group) as those that should drive future research initiatives (see Fig. 1).

CRITICAL CLINICAL PROBLEMS

1. Failure to provide adequate local control is an important cause of treatment failure in RMS (Wharam et al. 2004), and current local control interventions contribute substantially to long-term functional deficits and second cancers in those who achieve durable remissions (Punyko et al. 2005).

2. Most RMS tumors are systemic cancers with both early regional cancer growth and distant spread of cancer cells. Metastatic disease is a major contributor to death in primary and relapsed RMS (Pappo et al. 1995; Oberlin et al. 2008).

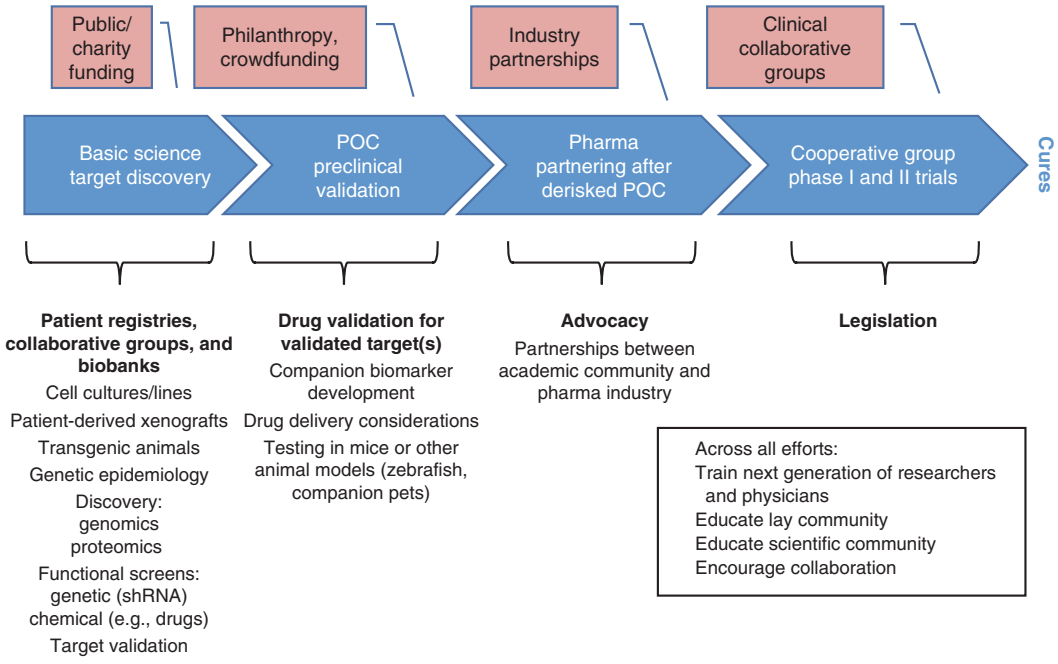

Figure 1. Rhabdomyosarcoma roadmap. Developing curative anti-RMS treatments will depend on collaborative efforts between academia, the pharmaceutical and biotechnology industries, and clinical collaborative groups (for example, the Children's Oncology Group [COG] and European Collaborative Groups), with support from public, charity, philanthropy, and crowd funding. The Sarcoma Alliance for Research through Collaboration and collaborative efforts between large sarcoma centers may allow access to adult RMS cases. Basic science discovery work will identify candidate targets and therapeutic agents for preclinical validation and proof of concept (POC) studies. Early collaborations with industry will be critical. The highest-priority targets/agents will be further developed, and early-phase clinical trials will be performed via existing collaborative group mechanisms. Across all these efforts, training of the next generation of physicians and researchers, education, and collaboration will be of critical importance.

3. Risk of treatment failure in pediatric RMS is stratified based on primary site, histology, and extent of disease. These stratification parameters do not currently include genotypic differences between fusion-positive and fusion-negative RMS or the full range of clinical, histological, and molecular variability seen across the RMS spectrum (Parham 2001; Xia et al. 2002; Missiaglia et al. 2012; Skapek et al. 2013).

4. 40% of all RMS tumors are diagnosed in adults, including individuals in the adolescent and young adult group (18–40 years of age) and older adults (>40 years of age) (Sultan et al. 2009; Egas-Bejar and Huh 2014). Adult RMS attracts very little attention, but overall survival in this age group is only 27% (Sultan et al. 2009). The clinical behavior, pathobiological identity, and therapeutic requirements of RMS in adults are largely unknown.

5. Several well-characterized, cancer-predisposing syndromes, including but not limited to Li Fraumeni, Neurofibromatosis type 1, and Costello syndrome (linked to germline mutations in *TP53*, *NF1I*, and *HRAS*), confer susceptibility to develop RMS (Kratz et al. 2011; Hettmer et al. 2014). However, the actual prevalence of cancer-predisposing germline mutations in people diagnosed with RMS and the therapeutic requirements of syndromic RMS are not known.

CRITICAL BIOLOGICAL PROBLEMS

1. *PAX-FOXO1*-positive RMS shows a higher propensity to metastasize and reduced survival after currently available multimodal treatment when compared with fusion-negative tumors (Williamson et al. 2010). Understanding of the mechanisms by which the fusion product impacts malignancy and how to interfere with these mechanisms or the fusion products themselves remains insufficient.
2. RMS tumors show variable skeletal muscle differentiation markers, and some tumors show a propensity to enter terminal myogenic differentiation after chemotherapy. Yet, chemotherapy-induced cytodifferentiation in RMS, more commonly observed in fusion-negative than in *PAX-FOXO1*-positive tumors, is generally incomplete (Coffin et al. 1997; Smith et al. 2002). A better understanding of the mechanisms that drive terminal myodifferentiation in RMS cells is needed.
3. Pathobiological heterogeneity across the RMS spectrum is poorly annotated, particularly with regard to the phenotypic differences between *PAX3-FOXO1*- and *PAX7-FOXO1*-fusion-positive RMS (Skapek et al. 2013), and the marked clinical, morphological, and genetic variability across the fusion-negative spectrum (Parham 2001; Xia et al. 2002).
4. The use of combinations of conventional cytostatic drugs revolutionized pediatric RMS treatment in the 1970s (Pratt et al. 1972). It is likely that the use of combinations of targeted agents will be critical to success in future anti-RMS therapeutic developments. Thus, rational approaches to prioritize and select drug combinations, for example by high-throughput screening or computational modeling, are needed.
5. RMS tumors originating at different body sites show markedly discrepant clinical behavior, and primary tumor site has long been recognized as a risk factor in RMS. For example, cure rates are substantially higher for RMS tumors arising in periorbital muscles compared with those originating in extremity muscles (Dagher and Helman 1999). Yet, very little is known with respect to contributions of the local microenvironment and cell of origin to RMS phenotype and behavior.
6. Phenotypically and functionally distinct subsets of cells with differential ability to repopulate tumors in secondary recipients, to self-renew, and to migrate were identified in models of fusion-negative RMS (Walter et al. 2011; Ignatius et al. 2012). However, potential differences in drug sensitivities between individual RMS cell subsets and their clonal evolution over time are not known. Moreover, the phenotype and differential tumor-repopulating/metastasis-initiating activity of cell subsets within the *PAX-FOXO1*-positive tumor cell pool remain poorly understood. Molecular characterization of specific, functionally relevant cell populations will likely open new therapeutic windows.

STRATEGIC CONSIDERATIONS

1. RMS is a rare cancer. Families affected by this cancer, patient advocates, clinicians, and scientists need to coalesce their efforts to garner sufficient support from funding sources and the pharmaceutical industry to support the development of curative therapies. Existing preliminary data, strategic considerations by relevant pediatric collaborative groups, and a deep clinical need may provide a fertile ground for partnerships between academic groups and industry (Sokolowski et al. 2014).
2. Insufficient access to fresh RMS tissue has hindered progress in RMS research. Moreover, there are few existing human RMS cell lines, and these lines are not widely distributed (Sokolowski et al. 2014). Tumor biology initiatives are needed to collect sufficient amounts of fresh tumor material from patients with RMS (both children and adults) to support tumor profiling efforts and the generation, long-term storage, and distribution of new cell lines and xenografts for experimentation.

3. Recent efforts to move new candidate drugs with anti-RMS activity into the clinic are primarily built on early-phase clinical trials designed to test single agents in heavily pretreated patients with bulky disease. Alternative trial designs should be considered to allow for (1) testing of rationally selected combinations of drugs, (2) evaluation of drug effects in a minimal residual disease setting (such as maintenance therapy), and (3) mandatory tumor biopsies before enrollment to facilitate identification of response markers.
4. New model systems and new perspectives, especially from the skeletal muscle biology community, may have substantial positive impact on RMS research.

PARTNERSHIPS BETWEEN ACADEMIA AND INDUSTRY

Access to primary human RMS tissue traditionally has been limited to academic medical institutions. However, the current funding environment significantly restrains academia's capacity to support deep genetic characterization and pharmacological interrogation of primary human RMS tissue and representative cell lines with the goal of identifying key regulatory pathways that may ultimately yield drug targets. Regrettably, the scarcity of samples also has dissuaded large pharmaceutical and biotechnology companies, the very entities with the capacity for large-scale genomic studies and screening, from investing in this area of drug discovery. To accelerate RMS research in the current funding environment, it is critical to establish sustainable collaborative partnerships between clinical centers, academic research groups, and industry to allow pharmaceutical and biotechnology companies to contribute their unique resources to generate comprehensive RMS data sets. The resulting data should be publically available and published in a timely manner. Such collaborative efforts will provide academic researchers with high-quality data allowing fundamental insights into RMS malignancy, and industry partners will be able to leverage this data to develop drug sensitivity testing and, perhaps, identify new drugs and new uses for previously established medicines.

One successful example for a productive partnership between academia and industry is the Cancer Cell Line Encyclopedia (CCLE) project, a collaborative project between Novartis and the Broad Institute. The CCLE provides a comprehensive set of data on 1000 cancer cell lines that has proven to be useful for cancer characterization and target identification (Barretina et al. 2012). Importantly, this resource was made public, even though Novartis assumed a significant portion of the cost. New cell lines, primary tissues, and primary tumor xenografts are currently being added, including samples representing rare tumor entities. Tumor prevalence is not a major criterion for inclusion, but a robust logistic framework allowing access to tumor material is needed.

The acquisition and transfer of primary tumor material between academic medical centers and industry partners could be facilitated by mutually agreed on standards between academic institutions and companies concerning patient consent forms, material transfer agreements (MTAs), and collection/distribution fees. Umbrella agreements between clinical groups and industry partners could circumvent the need to obtain individual MTAs for new samples, and thereby accelerate progress. An oversight committee representing all interested parties should supervise the collaborative effort to prioritize common interests and secure transparency.

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