SPECIAL SECTION

UPDATES in ONCOLOGY: PART II

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On the cover: As The Cancer Genome Atlas (TCGA) research papers were published on individual cancers – glioblastoma, breast, ovarian and others – and genomic data started to accumulate, investigators began noticing similarities across cancers, patterns in the chaos. The Pan-Cancer initiative, launched in 2012 as a next logical step in TCGA studies, allows scientists to find a new way to pool all of this information and see commonalities among disease types.
Updates in Molecular Pathology of Central Nervous System Gliomas in Adults

MICHAEL PUNSONI, MD; JOHN E. DONAHUE, MD; HEINRICH D. ELINZANO, MD; TIMOTHY KINSELLA, MD

ABSTRACT
Central nervous system (CNS) tumors are a heterogeneous group of neoplasms divided into two broad categories, glial and non-glial. Non-glial tumors are derived from such diverse structures as the pineal gland, meninges, germ cells, and hematopoietic cells, as well as metastases. Primary glial neoplasms, or those which originate from astrocytes, oligodendrocytes, or ependymal cells, include astrocytomas, oligodendrogliomas, ependymomas, and mixed gliomas. Each entity has a unique morphology and pattern of biologic behavior which portends a distinct prognosis and outcome. Individual outcomes show some variability based on tumor location and age of symptom onset; however, the underlying aggressiveness of the tumor often dictates the time course of the disease. With the advent and widespread use of fluorescent in-situ hybridization and polymerase chain reaction (PCR) techniques, molecular phenotyping of brain tumors has become mainstream and is now an integral part of patient care. The molecular genetics of CNS tumors is a rapidly growing field, and the volume of discoveries is growing at an ever increasing rate, compelling the need for updates in this exciting area of science.

KEYWORDS: Glioma, astrocytoma, oligodendroglioma, glioblastoma, 1p19q, MGMT, IDH1

INTRODUCTION
Within the broad spectrum of central nervous system (CNS) tumors, a number of common neoplasms carry a high rate of morbidity and mortality. In 2014, an estimated 22,850 adults in the United States were diagnosed with primary tumors of the brain and spinal cord. It was estimated that 15,320 of those affected would die of their disease that same year [1]. Furthermore, currently there are nearly 700,000 people in the U.S. living with a brain tumor [2]. While primary brain tumors are a diverse group of entities, with over 120 types of neoplasms, among the commonest, and one of the most studied, are the gliomas. Each neoplasm has a unique morphology and pattern of biologic behavior that shapes the clinical outcome of the individual. New discoveries in the molecular biology of these diverse brain tumors may offer advanced targeted therapies leading to decreased complication rates and improved quality of life.

Epidemiology
The incidence of CNS malignancies has been increasing. Most commonly, CNS tumors arise from glial cells, particularly astrocytes and oligodendrocytes. Gliomas account for approximately 77% of primary malignant brain tumors [3]. Most patients present between the fifth and seventh decade of life. High-grade tumors are more common than low-grade and present a high risk of morbidity and mortality. The World Health Organization (WHO) in 2000 formulated a classification system based on histologic findings that is used to stratify brain tumors into prognostic grades. High-grade gliomas (WHO grade III and IV) are most commonly seen in middle-aged to older adults, while grade II astrocytomas mainly affect younger adults. Management for low-grade neoplasms involves either observation or surgical excision, while high-grade tumors often require aggressive regimens involving chemotherapy and radiation after surgical debulking.

Pathology
CNS tumors are classified according to their predominant cell type. Therefore, most gliomas can be classified as astrocytic, oligodendroglial, or mixed oligo-astrocytic tumors. Furthermore, criteria such as atypia, mitoses, endothelial proliferation, and/or necrosis allow for application of the WHO grading scheme, which gives prognostic information based on tumor grade. Diffuse gliomas are devastating cancers due to their locally aggressive behavior, insidious infiltration into the adjacent brain tissue, and resistance to current treatment options. In addition, low-grade gliomas have a tendency to progress to anaplastic (grade III) gliomas, with anaplastic astrocytomas ultimately progressing to glioblastomas (WHO grade IV).

Molecular alterations, such as changes in chromosomal copy number, deletions, and duplications, are common events in gliomas. In recent years, molecular analysis of tumors has sharply increased in terms of available molecular studies and their impact on diagnosis and prognostication. Currently, it is believed that changes in gene expression and other genetic abnormalities may underlie transformation and progression of gliomas. Some of the most common molecular changes that are tested in gliomas are co-deletion of 1p19q, O6-methylguanine-DNA methyltransferase (MGMT) methylation, and Isocitrate dehydrogenase 1 (IDH1) mutation.
In those treated with temozolomide and radiotherapy, the median survival was 21.7 months compared to 15.3 months among those who only receive radiotherapy [7].

MGMT inactivation is an important marker of therapeutic application, commonly tested in GBM. GBM is a malignant primary brain tumor (WHO grade IV) that is often supratentorial. It may occur de novo (primary) or progress from lower-grade gliomas, such as an anaplastic astrocytoma (WHO grade III). GBM is an aggressive neoplasm that shows contrast enhancement on radiologic scans, often with large areas of peritumoral edema and mass effect on surrounding brain tissue. Grossly, the tumors are variegated with necrosis and hemorrhage. Microscopic features include hypercellularity with atypia, mitoses, endothelial proliferation, and either geographic or pseudopalisading necrosis. Due to their aggressive nature, early diagnosis with proper therapeutic management is essential.

In recent studies, MGMT was methylated in approximately 45-50% of glioblastomas and irrespective of treatment, MGMT promoter methylation was an independent favorable prognostic factor. Furthermore, a survival benefit was observed in that same group of patients following treatment with temozolomide and radiotherapy [7-8].

**1p/19q**

The combined loss of chromosomal arms 1p and 19q are commonly seen in oligodendrogliomas and are thought to be a marker of good prognosis. Oligodendrogliomas derive their name from their non-neoplastic counterpart, the oligodendroglial cell, a native support cell of the central nervous system. These tumors are diffusely infiltrating, well-differentiated (WHO grade II) gliomas, often occurring in adults. They tend to arise in the cortex and white matter of the cerebral hemisphere, with the majority of cases in the frontal lobes. Oligodendrogliomas are slow-growing tumors that are well-demarcated, non-enhancing mass lesions on radiologic scans. Grossly, the tumors may show mucoid changes, with areas of cystic degeneration, hemorrhage, and calcification. Microscopically, they are diffusely infiltrating glial tumors composed of monomorphic cells with uniform round vesicular nuclei, distinct small nucleoli, and a classic perinuclear halo which is an artifact of fixation (giving rise to the name “fried egg” cells). This is often accompanied by a delicate capillary (“chicken wire”) vasculature. These neoplasms infiltrate the adjacent cortex, specifically via perineural, perivascular, and subpial spread. Grade II tumors may progress to grade III tumors, which are known to have increased cellularity, nuclear atypia, and mitotic figures. A common molecular alteration in oligodendrogliomas is co-deletion of the 1p and 19q chromosomal arms. This combined loss is rare in astrocytomas and glioblastomas [GBM], however, it is seen in approximately 40–70% of classical forms of oligodendroglioma [4]. The incidence of 1p/19q loss is much lower in cases of mixed oligoastrocytoma [20–30%] [5]. Patients with anaplastic (grade 3) oligodendrogliomas whose tumors harbor the 1p/19q co-deletion have a more favorable prognosis if treated with chemotherapy and radiation therapy with an overall survival approaching 15 years compared to similarly treated patients whose tumors do not demonstrate the co-deletion in a large randomized clinical trial [overall survival of 7.5 years] [6]. While histopathology is the gold standard for diagnosis, molecular testing for the combined loss of 1p/19q is used as an adjunct for prognostication and treatment selection.

**MGMT**

MGMT (O\(^6\)-methylguanin-DNA-methyltransferase), a DNA repair enzyme located on chromosome 10q26, is involved in repairing damaged DNA from toxic effects of alkylation. In addition, this enzyme contributes to drug resistance of gliomas by protecting tumor cells from alkylating agents. MGMT promoter hypermethylation and epigenetic silencing lead to MGMT gene inactivity and loss of protein expression. In effect, methylation leads to susceptibility of tumor cells to the alkylating effects of agents such as temozolomide, thereby allowing for more effective treatment of high-grade gliomas that are methylated. A study by Heigi et al. showed a survival benefit among patients whose glioblastoma (grade IV) contained a methylated MGMT promoter.

**IDH1**

IDH1 (isocitrate dehydrogenase-1), a metabolic enzyme, is known to undergo mutations that are associated with gliomas and are thought to give prognostic implications to the diagnosis. The most common mutation affects codon 132, which causes conversion of arginine to histamine [R132H]. Immunohistochemical analysis can detect mutant IDH1, which is found in neoplastic gliomas cells and not in reactive gliosis. IDH1 mutations are frequently found in low-grade gliomas and to a lesser extent high-grade gliomas, including secondary GBM, and are associated with a favorable prognosis when compared to IDH1 wild-type [9-11]. A study by Ichimura et al. showed that codon 132 mutations were seen in up to 65% of oligodendrogial tumors, 54% of astrocytomas and 6% of glioblastomas [3% of primary GBM and 50% of secondary GBM] [12]. Using current WHO grading schemes, anaplastic astrocytoma (WHO grade III) has a better prognosis than GBM (WHO grade IV). Some research supports combining histologic grading of tumors with molecular phenotype, thereby creating a combined classification that gives a clear diagnostic and prognostic picture of each entity. For example, Hartmann et al. [13] proposed a sequence of favorable (median survival of 18–24 months) to clearly less favorable (median survival of 6–9 months) outcomes based on histologic and molecular status that ranged from anaplastic astrocytoma with IDH1 mutation, GBM with IDH1 mutation, anaplastic astrocytoma without IDH1 mutation and finally GBM without IDH1 mutation. Future randomized trials are needed to further elucidate the interaction and prognostic implications of this molecular alteration.
CONCLUSION

CNS tumors are a heterogeneous group of neoplasms with a wide array of histologic subtypes and an increasingly complex group of molecular abnormalities. Current standards of practice dictate that diagnosis is based primarily on histopathology, however, it is becoming increasingly common to order molecular studies to further characterize, classify, and prognosticate tumors. The vast catalogue of molecular alterations is steadily increasing, and further studies will be necessary to determine their significance in terms of diagnosis and prognosis.

References

12. Ichimura et al. IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro Oncol. 2009; 11 (4): 341-47.

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Molecular profiling helps define the phenotype of a cell and is designed to aid in early cancer detection, risk assessment, and targeted therapies. Profiles should be measurable across populations, useful for detection of cancer at an early stage, or assist in identification of high-risk individuals. As technology has developed, so has our ability to explore tumor biology down to the level of gene expression.

Endometrial cancer is the most common gynecologic malignancy in developed countries and the second most common in developing countries. Patients typically present with symptoms such as postmenopausal bleeding, which allows for detection at an earlier stage. Ovarian cancer is less common but carries a poorer prognosis given its typical late stage of diagnosis. This section will discuss developments in the treatment of endometrial and ovarian cancer at a molecular level. Considerable breakthroughs have been made in the area of poly ADP-ribose polymerase (PARP) inhibitors in BRCA mutated ovarian, fallopian tube, and primary peritoneal cancers. Epithelial cellular adhesion molecule (EpCAM) overexpression has been a target for the treatment of malignant ascites in ovarian cancer. There has been utility in targeting hormone receptors in the setting of recurrent endometrial cancer while the role of human epidermal growth factor 2 (HER2) and mTOR inhibitors shows promise but remains investigational.

POLY ADP-RIBOSE POLYMERASE (PARP) INHIBITORS
PARPs are a constitutive factor of the DNA damage surveillance network developed to cope with numerous environmental and endogenous toxic agents. In particular, the roles of PARP 1 and 2 in the base excision DNA repair pathway have been elucidated. Inhibition of the PARP enzyme leads to persistence of spontaneously occurring single-strand breaks and subsequent formation of double-strand breaks. PARP inhibitors were found to have anti-cancer activity both in vitro and in vivo in germline BRCA mutated cancer. BRCA1 and BRCA2 mutations act on the cellular level as tumor suppressor genes involved in double-stranded DNA (dsDNA) break repair. Using the concept of synthetic lethality, which is defined as the situation when mutation in either of two genes individually has no effect but combining the mutations leads to death, the use of a PARP inhibitor in patients with an existing BRCA mutation should disable the back-up repair mechanism thus leading to cell death. The implication is that targeting one of these genes in a cancer where the other is defective should be selectively lethal to the tumor cells but not toxic to the normal cells. In December 2014, olaparib [Lynparza ™] became the first PARP inhibitor approved by the FDA in the treatment of advanced ovarian cancer in patients with a known BRCA mutation who have received three or more prior lines of therapy. Approval is contingent upon the demonstration of positive results in two ongoing phase III clinical trials with olaparib limited to patients with BRCA mutations. Other PARP inhibitors such as veliparib have shown promising results in a phase II Gynecology Oncology Group (GOG) trial of patients with persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer with a BRCA 1 or 2 mutation. Niraparib and rucaparib are in ongoing clinical trials.

EpCAM
EpCAM is abundantly expressed on human cancers and EpCAM overexpression has been associated with a poor prognosis in patients with ovarian, breast, prostate and gallbladder carcinoma, both functioning as an oncogene and suppressing CD4+ T-cell-dependent immune responses. Tumor cells in malignant ovarian cancer-associated ascites have been shown to express EpCAM in 70–100% of cases, while the mesothelial cells lining the peritoneal cavity lack expression. Catumaxomab is a trifunctional monoclonal antibody with two different antigen-binding sites and a functional Fc domain: one binds to epithelial tumor cells via EpCAM and the other to T cells via CD3. Catumaxomab was evaluated as part of a phase I/II dose-escalating study for intraperitoneal application in patients with ovarian cancer who had EpCAM-positive tumor cells. Treatment with catumaxomab resulted in significant and sustained reduction of ascites. In April 2009, the European Union approved catumaxomab for the intraperitoneal treatment of malignant ascites in patients with EPCAM positive carcinomas where standard therapy was not feasible. It has not been FDA approved in the United States but is in clinical trials.

HORMONE RECEPTORS
The presence of progesterone receptors has been found to correlate with low-grade histology and overall more favorable outcomes. Receptor expression can be lost in either the

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primary tumor or in metastatic disease, its loss is associated with disease progression and decreased patient survival. Progestins have been employed to exploit the presence of progesterone receptors, however they paradoxically down regulate receptors when given continuously. The addition of tamoxifen to counter the progesterin induced down regulation has shown clinical benefit. Megestrol acetate [megace] and tamoxifen for the treatment of metastatic endometrial cancer is based on a Gynecologic Oncology Group (GOG) study where patients alternated three-week courses of megace and tamoxifen with an overall response rate of 27%, a median progression-free survival of 2.7 months and median overall survival of 14 months. This is a viable treatment option in patients who are not eligible for secondary cytoreduction or multidrug cytotoxic chemotherapy, which has demonstrated a response rate from 33–57%. Endocrine therapy has shown some, albeit limited, clinical utility in the treatment of relapsed epithelial ovarian, fallopian tube, and primary peritoneal cancers. Studies using tamoxifen, thalidomide, and letrozole have shown improvements in progression free survival [PFS] and overall survival [OS].

**HER2**

Although currently investigational, the role of human epidermal growth factor 2 (HER2) was shown to be a potential target in women with uterine serous carcinomas that overexpress HER2. A small GOG trial of 34 women with advanced or recurrent endometrial cancer showed no responses but 12 patients with overexpression of HER2 had stable disease. A clinical trial evaluating the role of trastuzumab in combination with chemotherapy in uterine serous papillary carcinoma is ongoing. Single agent lapatanib in one trial of patients with endometrial cancer showed that 3 out of 30 patients were progression free after 6 months, 1 patient had a partial response, and 7 had stable disease. Although these appear to be modest results, treatment options in the platinum resistant setting are needed. These women have a poor prognosis. The limited data suggest that the most likely response to second line treatment is stable disease at best and overall survival is usually less than a year.

**mTOR INHIBITORS**

Overactivation of the PI3K/AKT/mTOR pathway, a signaling pathway that plays an important role in cellular growth and survival, has been implicated in endometrial cancer pathogenesis. In a phase II study of previously treated recurrent or metastatic endometrial carcinoma patients, 25mg of IV temsirolimus showed a response rate of seven percent, however, 44 percent had stable disease, with a median duration of 5.1 to 9.7 months. These results have been promising enough to proceed with a clinical trial. GOG 86-P incorporates temsirolimus into one of three treatment arms for women with advanced, recurrent, or metastatic endometrial cancer not previously treated with chemotherapy. Results are anticipated in the near future.

**CONCLUSION**

Biomarkers for gynecological cancers, especially ovarian cancer, are the subject of multiple ongoing and planned clinical trials. Targeted therapies for these biomarkers are in rapid development and are hoped to improve the prognosis of women with gynecological malignancies by providing improvement in outcomes and treatment tolerability.

**References**


Genetics and diffuse large B-Cell lymphoma

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ABSTRACT
Diffuse large B-Cell lymphoma (DLBCL) is one of the most common and aggressive subtypes of non-Hodgkin’s lymphoma [NHL]. Gene expression profiling [GEP] studies have identified at least two distinct molecular subtypes of DLBCL termed as germinal center B-cell [GCB] and activated B-cell [ABC]. These molecular subtypes represent lymphomas that are driven by very different intracellular oncogenic signaling pathways which have prognostic value and could potentially be exploited for therapeutic benefit in future. There are other oncogenes, namely BCL-2, BCL-6 and MYC, which have been associated with the pathogenesis of DLBCL. Concurrent presence of two oncogenes is present in about 5% of DLBCL and it is termed “double hit lymphoma” [DHL]. DHL are associated with an aggressive clinical course and do not respond well to the standard DLBCL immune-chemotherapy regimen, RCHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone). Other aggressive therapeutic approaches including autologous bone marrow transplant have not shown any survival benefit in this subgroup of DLBCL patients. New strategies in development to address this resistance in DHL include the regimen DA-EPOCH-R [dose adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab]. Recent studies have shown increased sensitivity of DHL to DA-EPOCH-R chemotherapy and will likely be the new standard of care in this subset of DLBCL patients in the future.

KEYWORDS: Diffuse large B cell lymphoma, Activated B-Cell, Germinal center B-Cell, MYC, BCL-6 and BCL-2 oncogenes, RCHOP, DA-EPOCH-R.

Worldwide, Diffuse large B cell lymphoma represents the most common subtype of non-Hodgkin’s lymphoma [NHL] and it accounts for 30–40% of the newly diagnosed NHL cases.1 DLBCL typically behaves aggressively, quickly evolving over weeks to months and can be curable if treated early. However, with therapy DLBCL is curable in more than 60% of patients that are treated with combination immuno-chemotherapy, of which the most common regimen employed is RCHOP.2 Prognosis of DLBCL has traditionally been based on five patient characteristics: age, performance status, number of extra nodal sites of lymphoma involvement, stage and LDH value. Until recently, little was known regarding the impact of the molecular profile of malignant lymphoma cells on the treatment strategy and prognosis of patients with DLBCL. However, recent discoveries in the molecular biology of DLBCL have resulted in the identification of a subset of DLBCL which does poorly with standard RCHOP therapy.

Gene expression profiling [GEP] studies in DLBCL have identified at least 2 distinct molecular subtypes, germinal center B cell [GCB] and activated B cell [ABC].3 These molecular subtypes are believed to represent lymphomas arising from different stages of lymphoid differentiation. The GCB subtype arises from the centroblasts, whereas the ABC subtype arises from the plasmablastic cell just prior to germinal center exit.4 These molecular subtypes not only represent different intracellular oncogenic signaling pathways but are a result of the oncogenic pathway perturbations that result due to recurrent mutations, gains and losses of genetic material, and characteristic translocations. This molecular distinction has prognostic implications; the ABC subtype has an inferior outcome following R-CHOP chemotherapy compared to GCB subtype. One study has reported a 3-year progression-free survival [PFS] of 40% in ABC group vs. 75% in GCB group.5

The major oncogenic pathways that have been identified with varying frequencies in GCB DLBCL are c-rel amplification, EZH2 [histone methyl transferase] mutation, deletion of PTEN [causes activation of phosphatidylinositol 3 kinase/AKT/mTOR signaling pathway which are instrumental in cellular growth and metabolism].6 About 30% of GCB also have t 14,18 which causes increased expression of anti-apoptotic protein Bcl2.

In contrast, the pathogenic hallmark of ABC DLBCL is the constitutive activation of the NF-kB signaling pathway, which promotes cell survival, proliferation and inhibition of apoptosis. The activation of the NF-kB pathway is largely due to constitutive activation of the CBM signaling complex [formed by CARD11, BCL10 and MALT1]. The CBM complex can be activated by different genetic aberrations, 10% harbor activating mutations of CARD11 and in the remaining cases chronic active B-cell receptor signaling engages the CBM pathway. Chronic active B-cell receptor signaling is mediated through mutations in some B-cell receptor [CD79A or CD79B] and downstream kinases namely spleen tyrosine kinase [SYK] and Janus kinase 2 [JAK2].
kinase \(\text{[SYK]}\), Phosphatidylinositol3kinase \(\text{[PI3K]}\), bruton tyrosine kinase \(\text{[BTK]}\) and protein kinase C \(\text{[PKC]}\).\(^7\) Other mutations that have been observed in varying frequencies are mutations in MYD88, loss of TNFAIP3 which result in up-regulation and loss of inhibition respectively of NF-kB and Janus kinase pathways.\(^8\)

Along with the identification of intracellular oncogenic pathways, GEP studies have also identified molecular signatures related to the microenvironment of the tumor cells that are independent of these molecular subtypes. Stromal-1 signature reflects extracellular matrix deposition and infiltration of the tumor by macrophages and stromal-2 signature identifies tumors associated with high level of angiogenesis and high density of blood vessels. These molecular subtypes have been correlated with outcome; stromal 1 represents a prognostically favorable group compared to stromal 2 subtype.\(^5\) In addition to these, recurring lesions in the genes involved in immune recognition and antigen presenting functions have been recognized, suggesting that escape from immune surveillance plays an important role in the pathogenesis of DLBCL.\(^9\) There are, however, a subset of DLBCL whose biology cannot be explained by genomic events and transcriptional programs that are identified on the GEP, suggesting an additional layer of regulation. Recently somatic mutations in the epigenetic machinery have been identified suggesting the significance of epigenetic regulation in the normal B cell development and in lymphomagenesis. These epigenetic subgroups of DLBCL reflect the variability in DNA methylation, which has also been associated with clinical outcome.\(^10\)

The growing understanding of the molecular pathways in DLBCL has provided an opportunity to pharmacologically target these pathways to improve clinical outcomes in DLBCL. However, further work is needed to translate the recent discoveries to the clinical setting; this can be done by clinical trials, which support the use of tumor genetics in the formulation of therapeutic plans. There are many ongoing trials which are looking at agents that target various molecules in the oncogenic pathways of DLBCL. Immuno-modulatory agents which target the NFkB pathways like bortezomib and lenalidomide have been looked in non GCB DLBCL in retrospective studies. One study showed a higher response rate of 83\% vs. 13\% \(p<0.001\) and overall survival of 10.8 vs. 3.4 months \(p=0.003\) with the addition of bortezomib to chemotherapy in relapsed/refractory DLBCL.\(^11\) Similarly, studies using lenalidomide have demonstrated a high response rate in relapsed/refractory setting. Numerous phase III trials are ongoing which are looking at addition of these immunomodulatory agents with standard chemotherapy in non GCB DLBCL in first line setting.

One of the better-understood aspects of the pathogenesis in DLBCL is the alteration in the oncogenes and tumor suppression genes. Three such oncogenes are c MYC, BCL-2 and BCL-6, key regulation of cellular proliferation(c MYC) and apoptosis [BCL-2 and BCL-6]. BCL-2 and BCL-6 translocations are present in about 15 and 29\% of DLBCL respectively.\(^12,13\) The c-MYC translocation is present in about 5-10\% of DLBCL.\(^14\) Isolated presence of BCL-2 genetic aberration does not have independent prognostic value. Furthermore, it is controversial whether the c-MYC translocation alone has prognostic value in patients with DLBCL. However, recent studies have revealed that the impact of MYC is strongly influenced by BCL-2 or BCL-6. The presence of concurrent MYC and BCL-2 or BCL-6 translocation in patients with DLBCL, also known as “double hit lymphoma” [DHL], which has been associated with a very aggressive clinical course and an overall worse survival with standard R-CHOP chemotherapy. DHL occurs in nearly 5\% of cases of DLBCL.\(^15\)

Numerous studies have correlated the presence of MYC rearrangement and BCL-2 or BCL-6 with a poorer outcome in DLBCL treated with standard chemotherapy RCHOP.\(^15,16\) One example is a retrospective study by Akyurek et al investigating the impact of c-MYC, BCL-2 and BCL-6 rearrangements in 239 patients with DLBCL treated with RCHOP therapy. In patients with DHL, outcome was extremely poor with a median survival of 9 months and a 2-year overall survival (OS) rate of only 14\% vs. 78\% for non-DHL patients [Progression free survival, \(p = 0.003\) and OS, \(p < 0.001\)].\(^17\) Given the extremely poor outcome seen for this subset of DLBCL patients, clearly newer therapies are needed for DHL patients.

However, even though the double hit lymphomas have unacceptably poor cure rate with standard therapy, the optimal management approaches in this population of patients remains to be defined. As this subtype of lymphomas is rare, there are no large prospective studies evaluating the role of alternative forms of therapy in this poor prognosis group. One approach has been to use more aggressive chemotherapy treatments strategies, such as chemotherapy regimens used to treat the more aggressive form of NHL, Burkitt lymphoma, which consist of higher doses and more intensive chemotherapy cycles. However, one limiting factor is the high median age of patients with DLBCL, who due to co-morbidities may not be able to tolerate more aggressive regimens. One such attempt at this approach is the use of the aggressive chemotherapy regimen R-Hyper-CVAD [rituximab, fractionated cyclophosphamide, Adriamycin, dexamethasone alternating with high dose methotrexate, cytarabine and vincristine] frequently used to treat Burkitt lymphoma. However, the recent experience with this regimen in patients with DHL is not promising. For example, Li et al reported on a retrospective series of 52 patients with DHL treated with R-Hyper-CVAD regimen and found median OS in these patients was only 18.6 months.\(^18\) Other studies have reported similar poor outcomes with R-Hyper-CVAD in DHL patients.

Another approach to improve the outcome of DHL patients is to intensify therapy with the use of autologous bone marrow transplantation [BMT]. However, two retrospective
series investigating the role of autologous BMT did not demonstrate an advantage over standard immunochemo-
therapy regimens.\textsuperscript{19}

However, one very promising approach in patients with DHL is the use of prolonged infusional chemotherapy. One such regimen is dose-adjusted EPOCH with rituximab or DA-EPOCH-R. In this regimen the chemotherapy agents are similar to RCHOP with the addition of etoposide. However, the agents vincristine, Adriamycin and etoposide are given as continuous infusional therapy over a period of 4 days rather than the standard one day bolus regimen of RCHOP. Evi-
dence for this approach comes from in vitro data that sug-
gests that lymphoma cells of high proliferation rates may be more sensitive to a continuous exposure to chemotherapy rather than the more traditional bolus chemotherapy. Fur-
thermore, DA-EPOCH-R has been used successfully in the more aggressive c-MYC positive Burkitt lymphoma and it has been postulated that c-MYC positive lymphoma cells may show more susceptibility to infusional regimens such as DA-EPOCH then other DLBCL cells. Moreover, DA-EP-
OCH-R has been shown to be well tolerated in elderly patients. One other advantage to DA-EPOCH-R is that the
doses of the chemotherapy agents are adjusted up or down depending on absence or presence of associated toxicities, thereby, theoretically tailoring therapy to minimize toxicity while maximizing the doses of the chemotherapeutic agents.

One retrospective study published by Oki, et al has shown a superior rate of complete remissions (CR) with DA-EP-
OCH-R compared to R-CHOP in DHL patients. In this analysis, CR rates were found to be approximately 68% for DA-EPOCH-R compared to 20% for the more traditional RCHOP (p = 0.008).\textsuperscript{20} Preliminary data from a recent multi-
center Phase II study has shown promising activity with DA-EPOCH-R with progression free survival at 1 year of 87%.\textsuperscript{21} These data appear to show patients with DHL maybe more sensitive to DA-EPOCH-R chemotherapy versus the standard RCHOP regimen. However, longer follow-up of this prospective study will help us identify if this increased sensitivity results in a survival benefit for patients with DHL treated in this manner.

**CONCLUSION**

DLBCL is a very treatable and frequently curable NHL. GEP has helped us identify at least two subtypes of DLBCL (ABC and GCB) based on their different stages of lymphoid differ-
entiation. Each of these molecular subtypes of DLBCL has different intracellular oncogenic signaling pathways. ABC subtype is associated with poor prognosis and has poor outcomes with standard chemo immunotherapy compared with GCB subtype. Along with the cells of origin, molecular sig-
natures related to the microenvironment of the tumor cells and mutation in the epigenetic machinery have also been implicated in the prognosis of DLBCL which are indepen-
dent of these molecular subtypes. Given the poor prognosis

in these subtypes of DLBCL, numerous ongoing studies are looking at agents targeting the oncogenic signaling pathways. There is another known subtype of DLBCL called double hit lymphoma which has the presence of c-MYC rearrange-
ments in association with BCL-2 or BCL-6 rearrangements in patients with DLBCL. This delineates a population with an overall dismal prognosis for which new treatment strategies are needed. One promising mode of therapy is infusional therapy in the form of DA-EPOCH-R; however, we will need to await the results of prospective trials to ultimately deter-
mine if this regimen offers an improvement over standard approaches.

**References**

3. Sehn LH, Gascoyne RD. Diffuse large B-cell lymphoma: optimizing outcome in the context of clinical and biological hetero-
13. Shustik J, et al. Correlation between BCL6 rearrangement and outcomes in patients with diffuse large B Cell lymphoma treat-
14. Horn H, et al. MYC status in concert with BCL2 and BCL6 ex-
15. Green TM, Young KH, Visco C, et al. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus


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Genomics in acute myeloid leukemia: from identification to personalization

JAMES M. MARTIN, MD; ERIC S. WINER, MD

ABSTRACT
Acute Myeloid Leukemia (AML) is an aggressive bone marrow malignancy that is fatal if left untreated. Previous classification was strictly based on morphology, which gave little information in terms of prognosis or guide to treatment. Recent research has provided vital information into the chromosomal and molecular pathogenesis of leukemia development. The discovery of these abnormalities via proteomics and genomics have provided two key insights. First, these novel discoveries provide prognostic significance into the predictive result of chemotherapy. Second, these chromosomal and protein abnormalities have provided potential drug targets for new treatment modalities. This article will elaborate on many of these new molecular findings and discuss their implications on the treatment of AML.

KEYWORDS: Acute Myeloid Leukemia, AML treatment

INTRODUCTION
Over the past several decades, the field of oncology has transitioned from non-specific cytotoxic treatments to a more personalized approach to therapy. From the discovery of the Philadelphia chromosome in chronic myeloid leukemia to lung cancer’s EGFR gene mutations, every malignancy is unique—each with its own set of cytogenetic abnormalities and molecular mutations that provide each patient with an individualized collection of prognostic and therapeutic implications.

Acute myeloid leukemia, or AML, is no exception to this generalization. AML is a heterogeneous disease with many different pathogenic etiologies, clinical presentations and responses to treatment. Standard karyotypic analysis of the blast cells from AML patients indicate a large number of cytogenetic abnormalities among different patients, and it allows clinicians to stratify their patients into favorable-, intermediate- and unfavorable-risk groups, based on studies that looked at response to chemotherapy. For example, “favorable” cytogenetics include Inv(16), t(8;21) or t(15;17), although the later entity is classified as acute promyelocytic leukemia, which is treated as a completely separate entity altogether. “Unfavorable” cytogenetics include a complex karyotype (≥ 3 clonal chromosomal abnormalities), -5, -7, t(9;22) and many others. Included in the “intermediate”-risk cytogenetic class are those patients whose leukemic cells possess a normal karyotype (NK). Interestingly, NK patients have demonstrated a consistently variable response to standard treatment, which typically consists of induction chemotherapy, followed by either consolidation chemotherapy versus allogeneic hematopoietic stem-cell transplantation (allo-HSCT), based on clinical risk and individual patient factors. Thus, much of the recent research in molecular genetics within AML has the ultimate goal of better risk-stratifying these patients in order to provide better clinical outcomes.

Advances within the field of genomics have allowed for the detection of several different recurring genetic mutations in leukemic myeloblast cells of patient’s with normal cytogenetics. Typically, these genes increase signal transduction (leading to cellular proliferation) or affect transcription (causing impaired differentiation). While many of these molecular genetic changes do not impact clinical outcome, several mutations have been shown to significantly alter a patient’s ability to achieve a complete remission (CR), worsen the chance of relapse or effect overall survival (OS). Moreover, these studies have allowed clinicians to further risk-stratify NK patients, which subsequently may alter treatment decisions or make the patient eligible for novel small molecule inhibitors. Pertinent mutations that will be discussed in further detail below are FLT3-ITD, NPM1, CEBPA, DNMT3A, IDH1/2, TET2, ASXL1 and RUNX1, as well as the novel treatments available for these patient populations.

FMS-LIKE TYROSINE KINASE 3 (FLT3)
FLT3 (also known as CD135) is a tyrosine kinase receptor that is expressed on the surface of many hematopoetic progenitor cells. It activates signal transduction and is involved in cellular proliferation and differentiation. The FLT3-ITD (internal tandem duplication) mutation can be found in approximately 28-30% of NK AML. It has been shown to be an independent risk factor for poor outcome, specifically increased relapse rate and decreased OS. The adverse effect demonstrated by FLT3-ITD has suggested that NK patients with this mutation may be better classified as adverse-risk, allowing for clinicians to consider more aggressive therapy, such as allo-HSCT, based on known poor outcome with conventional chemotherapy. A second FLT3 mutation demonstrates a point mutation in the tyrosine kinase domain (FLT-TKD), although its prognostic significance is more clinically variable.
Not surprisingly, FLT3-ITD has become a novel target with several different therapeutics currently involved in clinical trials. The most widely studied is sorafenib, a non-specific tyrosine kinase inhibitor approved for renal cell carcinoma, hepatocellular carcinoma and thyroid cancer. Sorafenib has been proven safe and effective in relapsed/refractory AML. It also may have some possible benefit for maintenance therapy after allo-HSCT. Midostaurin, another relatively non-selective FLT3-inhibitor, was shown in a phase Ib clinical trial to have high (92%) CR in younger patients with newly-diagnosed AML when combined with standard chemotherapy. The phase IIb trial by the same group demonstrated a decrease in blast count with midostaurin treatment, revealing better responses in the FLT3-mutated population compared to the FLT3 wild type population. A phase III trial (CALGB 10603) is currently ongoing. Finally, quizartinib, a highly-selective FLT3-inhibitor has proven effective in relapsed/refractory AML with a 53% response rate in FLT-3 positive patients, and is currently being tested in combination with standard therapy [NCT01390337].

**NUCLEOPHOSMIN 1 (NPM1)**

The NPM1 gene encodes for a protein [nucleophosmin] with many functions, including nuclear transportation and regulation of tumor suppressor genes. It can be found in 45-64% of all NK AML, and approximately 33% of all cases of AML. Mutations in NPM1 lead to abnormal cytoplasmic localization of the protein that can be diagnosed with immunohistochemistry on bone marrow samples. While the NPM1 mutation has been shown to often be associated with other mutations, such as FLT3-ITD, DNMT3A and IDH1/2, it has been associated with a favorable prognosis with higher relapse-free survival and OS in patients without co-occurring FLT3-ITD mutations. This benefit is seen not only in younger patients, but also in older patients, even those over the age of 70. Thus, patients with NPM1 mutations, without FLT3-ITD or other poor prognostic features, can likely pursue standard chemotherapy (with induction followed by consolidation), without necessarily needing allo-HSCT.

### Gene Mutation

<table>
<thead>
<tr>
<th>Gene Mutation</th>
<th>Effect</th>
<th>Incidence</th>
<th>Potential Therapy Implications</th>
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<tbody>
<tr>
<td>Fms-like tyrosine kinase 3 (FLT3)</td>
<td>Tyrosine kinase receptor that activates signal transduction and cellular proliferation</td>
<td>Approximately 30% NK AML</td>
<td>Phase II success with TKI (sorafenib, midostaurin, quizartinib); clinical trials ongoing</td>
</tr>
<tr>
<td>Nucleophosmin 1 (NPM1)</td>
<td>Protein involved in transportation and regulation of tumor suppressor genes</td>
<td>45-64% NK AML</td>
<td>Potentially favorable prognostic factor</td>
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<tr>
<td>CCAAT/Enhancer binding protein alpha (CEBPA)</td>
<td>Transcription factor involved in differentiation of myeloid precursors</td>
<td>10-18% NK AML</td>
<td>Potentially favorable prognostic factor</td>
</tr>
<tr>
<td>DNA-methyltransferase 3A (DNMT3A)</td>
<td>Enzyme involved in epigenetic modification (methylation) of DNA</td>
<td>25% NK AML</td>
<td>Patients may benefit from high-dose anthracyclines in induction therapy; 5-azacytidine/decitabine currently used for MDS and being studied for AML</td>
</tr>
<tr>
<td>Isoctirate dehydrogenase 1/2 (IDH1/2)</td>
<td>Mutant enzymes that produce d-2-hydroxyglutarate, an oncometabolite that interferes with histone function and leads to oxidative stress</td>
<td>10-14%, 10-19% NK AML, respectively</td>
<td>Phase I trials ongoing with IDH1/2 inhibitors, AG-120 and AG-221</td>
</tr>
<tr>
<td>Additional sex combs-like 1 (ASXL1)</td>
<td>Protein involved in chromatin modification and remodeling</td>
<td>3% NK AML &lt; 60 years of age; 16% NK AML &gt; 60 years of age</td>
<td>Poor prognostic factor</td>
</tr>
<tr>
<td>Ten-eleven-translocation-2 (TET2)</td>
<td>Enzyme involved in epigenic modification (deoxygenation) of DNA</td>
<td>18-23% NK AML</td>
<td>Poor prognostic factor</td>
</tr>
<tr>
<td>Runt-related transcription factor 1 (RUNX1)</td>
<td>Transcription factor involved in hematopoiesis</td>
<td>8% NK AML &lt; 60 years of age; 16% NK AML &gt; 60 years of age</td>
<td>Poor prognostic factor</td>
</tr>
</tbody>
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NK AML, normal karyotype acute myeloid leukemia; TKI, tyrosine kinase inhibitors.
CCAAT/ENHANCER BINDING PROTEIN ALPHA (CEBPA)

CEBPA is a gene that encodes for CCAAT/enhancer binding protein alpha (CEBPα), a transcription factor protein that binds to promoter regions of DNA leading to growth arrest and cellular differentiation of myeloid precursors. The mutation can be found in 10-18% of adult patients with NK AML, and is also associated with the 9q deletion. Like NPM1, patients with CEBPA gene mutations, without concomitantly occurring FLT3 mutations, have a decreased relapse rate and increased OS. Patients can have either one or two allelic mutations, and patients with two mutations have been shown to carry the improved prognosis. This mutation has already been incorporated into the European LeukemiaNet classification, although a therapeutic target is not currently known at the time of writing this article.

ISOCITRATE DEHYDROGENASE 1/2 (IDH1/2)

IDH1 and IDH2 are homologs of the enzyme isocitrate dehydrogenase occurring in the cytosol and mitochondria, respectively. These mutant enzymes catalyze the conversion of α-ketoglutarate to 2-oxoglutarate (2-HG), an oncometabolite. Mutations in IDH1/2 have been known to occur in various types of brain tumors, but have also been found in approximately 10-14% [IDH1] and 10-19% [IDH2] of NK AML. Both mutations have been found to carry poorer prognosis in patients with NK AML. These mutations and 2-HG are currently of great clinical interest, as they may be used monitor treatment response and become targets of novel therapies. Currently, there are inhibitors to IDH1 [AG-120, Agios, Cambridge] and IDH2 [AG-221] under evaluation for AML. Although the pharmacokinetics data from the phase I trial of AG-120 has been presented, the early studies for AG-120 [NCT02074839] and AG221 [NCT01915498] are ongoing.

ADDITIONAL SEX COMBS-LIKE 1 (ASXL1)

ASXL1 encodes for a protein that is involved in chromatin modifications and remodeling. Mutations in this gene have been studied in other hematologic malignancies, but have only recently been identified as an adverse prognostic indicator in AML. ASXL1 mutations occur in approximately 8-13% of patients with NK AML, although it has been demonstrated more frequently in abnormal karyotype intermediate-risk cytogenetics, such as trisomy 8, and MDS-related AML. It is also notable that ASXL1 mutations occur with a 5-fold higher frequency in patients over 60 years of age compared to patients younger than 60. Patients with ASXL1 mutations demonstrate both a lower CR and OS.

DNA-METHYLTRANSFERASE 3A (DNMT3A)

Genomic studies have identified a mutation in the DNMT-3A gene that has become a significant negative prognostic indicator for AML. This key enzyme is involved in epigenetic regulation via DNA methylation. There are two mutations with DNMT3A – one which affects codon R882 that has a worse prognosis for older patients, while other DNMT3A mutations are related to a worse prognosis in younger patients. Mutations in this gene occur in approximately one-quarter of patients with NK AML and are associated with an OS of 12.3 months compared to 41.1 months. These patients were more likely to be older, and also more commonly had concomitant mutations in NPM1, FLT3, and IDH1 as well as more frequently noted to be in the NPM1-FLT3 low-risk group.

TEN-ELEVEN TRANSLOCATION-2 (TET2)

TET2 is an oncogene that has been identified in myelodysplastic syndromes and 18-23% of de novo NK AML. It has been strongly associated with secondary AML, older AML patients and a higher pretreatment white blood cell count. A recent CALGB study demonstrated that in NK patients, who would be otherwise categorized as having a favorable mutational profile, the presence of the TET2 mutation led to lower response rates and a high risk of relapse or death. This may lead clinicians to suggest more aggressive treatment regimens, rather than standard chemotherapy, which would typically be offered to patients with an otherwise favorable risk.

RUNT-RELATED TRANSCRIPTION FACTOR 1 (RUNX1)

The RUNX1 mutation involves the α-subunit of core binding factor, which takes part in the differentiation of hematopoietic progenitor cells. It is associated with NK AML in 8% of patients under the age of 60, yet 16% of patients over 60. The RUNX1 mutation was associated with inferior CR rates [47% versus 77%] and shorter disease-free survival. Most importantly, patients with RUNX1 mutations had a markedly decreased 5-year overall survival rate, with RUNX1 mutated patients at 2% while non-mutated at 30%. RUNX1 mutations were also found with concomitant mutations in ASXL, MLL, and IDH2, but less likely to be present in patients with NPM1 and CEBPA.

CONCLUSION

While many recurring mutations have been identified, adequate studies have yet to determine which specific mutations have clinical relevance. Moreover, several studies have demonstrated the frequent co-occurrence or mutual exclusivity of several mutations, which leads to further questions regarding which particular mutations may be oncogenic initiators versus clonal variations or downstream mutations. Although multiple mutations have been described here and in the literature, only NPM1 and FLT3 have clinical studies available, while IDH1 and IDH2 have small
molecule inhibitors in clinical trials. As genomic testing becomes commercially available, newly diagnosed patients with AML should have molecular studies performed, in addition to the standard karyotype analyses. Genomic advances have allowed clinicians to more appropriately risk-stratify patients, and future utilization of novel targeted inhibitors will likely lead to the development of successful personalized treatment plans for patients with AML.

References


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Melanoma Genomics and Immunotherapy

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ABSTRACT

Over the last decade the molecular characterization of melanoma has progressed. Since a majority of melanoma cases arise from repeated intermittent ultra violet radiation (UVR) exposure, the role of UVR has been evaluated extensively. Recent work has identified two mechanisms in which the carcinogenesis of melanoma may result; Ultra violet radiation has been demonstrated to lead to down regulation in immune responses and result in pyrimidine dimerization. Given these links and more significant immunogenic antigen profile of melanoma, as compared to other malignancies, there has been significant therapeutics breakthroughs based on these molecular pathways.

KEYWORDS: Tumor profiling, Melanoma, Immunotherapy

MELANOMA RATES ARE RISING RAPIDLY

Over the past 30 years, the incidence has doubled among women and tripled among men. More than 75,000 new cases of invasive melanoma will be diagnosed in the US in 2015. Melanoma claims 8,776 lives annually. The cost of treatment for melanoma is $3.3 billion per year and continues to rise.1

In the majority of cases, melanoma arises from repeated intermittent sun exposure especially in individuals with a history of multiple severe sunburns in childhood and adolescence. Melanoma is associated with a more immunogenic antigen profile compared to other malignancies. Ultra Violet radiation exposure leads to down regulation in immune responses. The absorption of UV radiation leads to a release of mediators that can affect antigen-presenting cells locally and systemically. This generates antigen specific T-cells capable of regulating immunity. In addition, UV exposure can lead to pyrimidine dimerization on DNA. If these products are not removed by cellular repair activities, after DNA replication, they may cause mutations.2

Regardless of the cause of the rise there has been an increase in survival due to the development of new treatments. The new treatments are targeted therapies which have emerged from advances in genetic profiling of molecular targets. Three key molecular pathways have been identified as highly deregulated in melanoma and include: mitogen-activated protein kinase (MAPK), PI3K/AKT and CDKN2A [p16] pathway.3,4

I. MAPK Pathway

Advances in melanoma have emerged from an increased understanding of melanoma biology and signaling pathways. The mitogen activated protein kinase (MAPK) pathway is a signaling cascade that has been studied extensively in melanoma. Deregulation occurs as a result of acquired mutations along this pathway.

This cascade includes several upstream signals that are funneled through RAF, MEK and ERK. Downstream molecules such as phosphatases, communicate with higher levels in the pathway to appropriately reduce signaling in normal cells. In melanoma, cells with an activating BRAF mutation, feedback is inhibited, which keeps the pathway switched on. The most prevalent are the BRAF mutations seen in at least 45% of cases. RAS mutations have a prevalence of 15% and the surface receptor tyrosine kinase, c-Kit is rare with 2-3% prevalence. Downstream mutations include amplification of CDK4 [30%], CCND [10%] and the survival oncogene microphthalmia associated transcription factor [MITF] [10%]. 5,6

BRAF is a serine/threonine kinase, a component of the MAPK pathway downstream of RAS. BRAF activation is a very strong signal, when it is activated it triggers the phosphorylation of MEK. BRAF mutations occur early in melanogenesis. About 50% of all melanomas have BRAF mutations. V600E mutations represent 80-90% followed by V600K, V600D and V600R that account for 5-15% of all BRAF mutations. These are more common in intermittently sun exposed skin and superficial spreading melanomas.

BRAF inhibition is associated with a robust clinical...
response. Vemurafenib and Debrafenib inhibit cells with a BRAF mutation. Immunohistochemistry of tumor biopsies showed a positive association between tumor response and the percentage decrease in cytoplasmic phosphorylated ERK supporting the proposed mechanism of action. A 3-year overall survival rate of 30% was observed in long-term follow-up with a significant improvement over standard therapy, Dacarbazine (DTIC). BRAF inhibition response is heterogeneous and rapid but resistance to therapy is seen within a year.

Within the MAPK pathway 37% of patients have a secondary RAS mutation that co-exists with the BRAF mutation. Alternative splicing, which allows BRAF to dimerize and increase signaling occurs in 20% of patients. BRAF amplification, which may produce a 50-fold increase in BRAF copies, occurs in 30% of patients. Downstream mutations in MEK 1/2 (2%) and cdk4 [11%] are less common. 7,8 The pattern of acquired BRAF inhibitory resistance follows a branched rather than linear path. This heterogeneity supports the rationale for early use of combined BRAF and MEK inhibitors in pursuit of a durable response. When resistance-related disease progression occurs while taking a BRAF inhibitor a secondary response may occur when a MEK inhibitor is added. 9,10,11

Debrafenib, a BRAF inhibitor, used in combination with Trametinib, a MEK inhibitor, is associated with an improved 12-month OS of 72% vs 63% in the monotherapy group and a MPFS (Median Progression Free survival) of 11 months vs 7.3 months. The combination therapy delays resistance and is associated with lower treatment-related toxicity. 12

II. PI3K/AKT: RAS and PTEN

The RAS proteins belong to a family of p21 proteins. These are all part of a complex network of pathways resulting in the release of nuclear transcription factors leading to expression of genes involved in mitogenesis and apoptosis. 7,8 Three closely related proto-oncogenes encoding the HRAS, KRAS and NRAS are found in mutated forms in human tumors. NRAS mutations are common in myeloid leukemia and melanomas. About 20% of melanomas have NRAS mutations. NRAS and BRAF mutations are mutually exclusive. There are no successful therapeutic targets for mutant NRAS mutant melanomas.

The pathways that could be targeted in NRAS mutant melanoma include MEK, P13K/m-TOR and cell related targets. Monotherapy with MEK inhibitors was associated with partial responses of 20% in this group of patients.

The PTEN gene is located on chromosome 10. Mutations in PTEN are found in 10%-20% of melanomas. PTEN has lipid phosphatase activity, which prevents formation of intracellular signaling molecules required for conformational change, which results in activation of the AKT protein kinase family. Activation of AKT pathway suppresses apoptosis through phosphorylation and inactivation of pro-apoptotic proteins. DNA copy gain of the AKT3 locus is found in 40%-60% of melanomas and results in activation of the AKT protein kinase. AKT3 expression correlates with melanoma progression. Thus, inactivation of PTEN allows signaling through the AKT pathway, which contributes to cell growth and anti-apoptosis. Evidence suggests that there is cooperation between loss of PTEN and BRAF mutations. 14

Adaptive responses by the tumor are reflected in pathway alterations contributing to acquired resistance to therapy. In more than half of the cases, the MAPK pathway, previously blocked by the BRAF inhibitors is reactivated, and p13k-PTEN-AKT alteration is involved in 4% of resistance development.

III. AKT: CDKN2A/p16

The CDKN2a gene products, p16 [tumor suppressor molecule] and p14 are cell cycle regulators that are frequently nonfunctional, especially in tumors arising from chronically sun-damaged skin. CDK4 and CDKN2A mutations are more common in acral and mucosal melanomas. The p16 protein binds to CDK4/6 kinase, blocking phosphorylation of the retinoblastoma protein and therefore leading to cell cycle arrest and inhibition of melanogenesis. Dysfunction in the proteins involved in this pathway promotes cell growth. P14 protein inhibits oncogenic activity of Bax/bcl-2 proteins that are responsible for effective apoptosis and are associated with resistance to anticancer therapy. 15

OTHER MOLECULAR PATHWAYS

C-Kit is a receptor tyrosine kinase [RTK] activated by binding of a stem cell factor. C-Kit plays an important role in proliferation, development, and survival of melanocytes, hematopoietic cells and germ cells. C-Kit mutations or amplifications activate a signal transduction pathway that ultimately leads to melanogenesis.Mutations in C-Kit are found in mucosal, acral and permanently exposed skin melanomas. C-Kit mutations or copy number gains are found in 39% of mucosal melanomas and 36% of acral melanomas. C-Kit mutations have also been shown to occur in up to 88% of oral mucosal melanomas and 15% of anal melanomas. Acral melanomas and mucosal surfaces appear to be the most aggressive subtypes. C-Kit mutations are rare in melanoma but inhibitors such as Imatinib and Nilotinib have shown promising activity in patients with exon 11 and 13 mutations. Phase II data showed overall disease control rate of 50%. 16 The presence of NRAS mutations is associated with resistance to Imatinib in the C-Kit mutant melanomas. BRAF mutations are less frequent in these melanomas.

Uveal melanoma arises from melanocytes of the choroid, ciliary body, and iris. Unlike cutaneous melanomas, which more frequently metastasize to lymph nodes, lung and brain, uveal melanomas often spread to the liver. Metastatic disease is aggressive andwith no effective treatment options for this group of patients. GNAQ and GNA11 pathway dysregulation appears to be responsible for the development of uveal
melanomas. GNAQ and GNA11 are genes that up-regulate the MAPK pathway when constitutively active. Mutations in GNAQ and GNA11 are mutually exclusive and found in more than 80% of uveal melanomas. These mutations are potential targets of therapy through blockade of the mutated proteins or other signaling molecules downstream in the same signaling pathways.7,16

IMMUNOTHERAPY

Over the past decade, new developments in T cell immunology have changed treatment algorithms and led to significant improvements in survival in patients with metastatic melanoma. T cell activation and proliferation are upregulated or down-regulated by checkpoint proteins that originate during distinct phase of the T cell response. Several immune checkpoint molecules have been identified many of which are co-expressed on cancer specific T cells. T cell activation occurs away from the target tumor Cytotoxic T lymphocyte associated molecule-4 (CTLA-4). CTLA-4 is a negative regulatory molecule that is translocated to the surface of the T cells after activation. This molecule ligates receptor B7 on antigen presenting cells (APC) and down-regulates T cell responses within 72 hours after activation. Blocking the ligation of CTLA-4 to the APC permits proliferation of activated cells.17

Ipilimumab

Ipilimumab is a monoclonal antibody to CTLA-4 approved for treatment of metastatic or unresectable melanoma. This therapy is associated with a disease control rate of about 30% and a 2-year survival of 29%, with a long-term durable response achieved in 20% of patients. There are no clear markers to predict response to therapy.18

Anti PD1/L1 immune blockade

Cytokines activate T cells that subsequently proliferate and migrate to the tumor microenvironment, where terminal inhibition of activated T cells occurs. PD1 and its ligand PD L1 which is up regulated on the tumor cells upon T cell recognition, are important immune checkpoint proteins. Whereas anti CTLA-4 inhibition occurs at the lymph node checkpoint, PD 1 blockade occurs in the tumor microenvironment.

Nivolumab is an anti PD1 humanized monoclonal antibody approved for treatment of metastatic melanoma in patients who failed prior therapy with Ipilimumab or other targeted therapies. Nivolumab achieved response rates of 32% with durability ranging from 2.6–10+ months. Pembrolizumab is another IgG4 human programmed death receptor-1 engineered blocking antibody approved for patients with unresectable or metastatic melanoma and disease progression following Ipilimumab or BRAF targeted therapy. Overall response rate was 24% with ongoing responses 6 months or longer.19

PD-L1 is an inducible ligand with several immune system functions. When expressed on tumor cells this protein down regulates the immune response. Histologic studies have shown that PD-L1 expression on the leading edge of the growing tumor further supporting the role that PD-L1 plays in the suppression of the immune system.19 Treating patients with compounds known to induce tumor PD-L1 has been suggested and this strategy is currently being investigated in clinical trials.

PD-L2 is a homologue ligand of PD-L1 both of which have normal physiologic functions in humans. Dendritic cells and macrophages express high levels of PD-L2 after immune challenge. Therefore specificity of PD antibodies is important. Results from a phase 1 study on anti PD-L1 antibodies suggested similar responses to that achieved with anti PD-1 antibodies; an overall response rate of 32% was observed and several patients achieved durable responses. Patients with >5% cell expression of the PD-L1 had better disease control rates [80%], compared to those with <5% PD-L1 staining.20

OTHER IMMUNE MODULATORY RECEPTOR TARGETS

<table>
<thead>
<tr>
<th>Receptor Target</th>
<th>Effect</th>
</tr>
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<tbody>
<tr>
<td>GITR (glucocorticoid induced TNF receptor)</td>
<td>Increases expansion of the T cell population</td>
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<tr>
<td>OX40</td>
<td>Plays a role in T cell regulation</td>
</tr>
<tr>
<td>CD137</td>
<td>Produces CD8 T cell activation, decrease in B natural killer cells and CD4 T cells</td>
</tr>
<tr>
<td>LAG-3</td>
<td>Inhibitory signaling molecules expressed in conjunction with PD-1 on CD8 T cells</td>
</tr>
<tr>
<td>TIM-3</td>
<td>Inhibitory signaling molecules expressed in conjunction with PD-1</td>
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CONCLUSIONS

Molecular differences among melanoma are extremely valuable for best therapeutic options and targets. Despite the recent advances in this field most patients ultimately relapse because of resistance. Many studies are underway investigating mechanisms and pathways to prolong treatment response and combat resistance.

References

6. Albino AP, Nanus DM, Mentle IR, et al. Analysis of ras onco-
genes in malignant melanoma and precursor lesions: correlation of point mutations with differentiation phenotype. Oncogene. 4:1363-1374; 1989


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