S5-FUTURE DIRECTIONS IN THERAPY OF ADVANCED THYROID CANCER.

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THE EXPERIENCE WITH MULTIKINASE INHIBITORS:
The primary emphasis in experimental therapeutics of advanced thyroid cancer over the past decade has been on testing the effectiveness of ATP-competitive multikinase small molecule inhibitors. These are thought to act mostly by inhibiting receptor tyrosine kinases such as VEGFR and PDGFR, which play an important role in tumor neovascularization. Although sorafenib is the only member of this class that is FDA-approved for treatment of follicular cell-derived thyroid cancer, several others, such as pazopanib, lenvatinib, motesanib and vandetanib, have also shown clinical benefit, manifesting as RECIST-confirmed partial responses and extension of progression-free survival (1). It is unclear whether these compounds act entirely through their effects on the tumor microenvironment, or whether they also exert direct growth inhibitory effects on the cancer cells, and if so, through which mechanisms.

Sorafenib inhibits RAF kinase activity \textit{in vitro}, but is unlikely to achieve therapeutically significant inhibitory effects on oncogenic BRAF in patients because of the narrow therapeutic window of the compound. In this respect, one key lesson from the experience of treating patients with metastatic BRAF-mutant melanomas is that clinical efficacy requires inhibiting BRAF kinase activity in a profound and sustained manner, which can be achieved with compounds that are highly selective, such as vemurafenib (2) or dabrafenib. By contrast sorafenib was ineffective as a monotherapy in patients with BRAF-mutant melanoma (3).

Other drugs, such as vandetanib and lenvatinib, have been proposed to have cancer cell intrinsic inhibitory effects by targeting EGFR. There is no evidence that EGFR is a primary driver of the disease, as EGFR mutations and gene amplification are rarely, if ever, present (4). Moreover, a small trial of gefitinib showed very modest effects in patients with metastatic thyroid cancer (5).

Despite evidence for clinical benefit, we still don’t know whether multikinase inhibitors prolong overall survival of patients with advanced thyroid cancer. One corollary of the experience with this class of compounds in thyroid cancer is that they do not truly represent examples of personalized therapies, since the genetic characteristics of the tumor do not predict clinical responses. The specific proteins or pathways targeted by these drugs that are critical for clinical activity have not been clearly established. Although these agents inhibit overlapping molecular targets, different inhibitors within this class can still induce responses in the salvage setting following sorafenib failure (6), possibly due to the existence of non-redundant targets and/or differences in the therapeutic window for each drug. Promising results are being reported with combinations involving multikinase inhibitors and other targeted agents (e.g., mTOR inhibitors), without definitive insights into how these agents interact to augment clinical responses. Without a better understanding of their mechanism of action, it will continue to be difficult to envision how to rationally design new treatment paradigms with these compounds.
THE ROLE OF CYTOTOXIC CHEMOTHERAPY AGENTS:
The emerging role of molecular targeted therapies for treating thyroid cancer raises the question regarding the relevance of cytotoxic chemotherapy agents for managing this disease. Until the recent FDA-approval of sorafenib, doxorubicin was the only systemic agent approved for the treatment of thyroid cancer (7). The concern regarding cytotoxic drugs such as doxorubicin, paclitaxel, and cisplatin is that the limited published studies report only modest response rates and short durations of therapeutic benefit. Nonetheless, these drugs are still acceptable options for patients who cannot tolerate treatment with multikinase inhibitors or who have relative contraindications to the use of anti-angiogenic therapies, such as the existence of active bleeding (e.g., hemoptysis).

Anaplastic thyroid cancer is an aggressive, rapidly progressive disease for which multikinase inhibitors have no significant clinical efficacy (8). Currently, cytotoxic agents remain the only viable systemic option (9). Given the genetic complexity of anaplastic thyroid cancer and the clinical need to induce tumor responses rapidly, this is the thyroid cancer subtype in which cytotoxic chemotherapy will likely continue to be a relevant part of future investigational approaches, primarily in combination with molecular targeted therapies. For instance, recent gene expression profiling of anaplastic thyroid cancers in mouse models revealed the presence of a deregulated “mitotic signature” in addition to the canonical “driver” mutations that result in constitutive MAPK pathway activation (e.g., mutant \textit{BRAF}, \textit{RAS}, and others) (10). This biology suggests that combinations of a cytotoxic that can elicit mitotic arrest (e.g., paclitaxel) with a MAPK pathway targeted therapy may be worth testing for anaplastic thyroid cancer.

Cytotoxic chemotherapy may also be used as a radiosensitizer for external beam radiation therapy applied to locally advanced or recurrent thyroid cancer. Retrospective studies show that radiation combined with doxorubicin can elicit effective locoregional control for locally-advanced, follicular-cell derived thyroid carcinomas (11) and to a lesser extent anaplastic thyroid cancer (12). An area of active investigation is how molecular targeted agents alone or in combination with cytotoxic chemotherapy may elicit more effective and durable locoregional control when administered concurrently with radiation therapy.

TARGETING THE GENETIC DRIVERS OF THYROID CANCER:
The Cancer Genome Atlas (TCGA) program recently completed a comprehensive genomic analysis of approximately 500 papillary thyroid cancers (PTC), which largely confirms prior studies regarding the frequency of the key driver mutations in the disease: \textit{BRAF} 57%, \textit{RAS} 12% and fusion oncogenes (\textit{RET/PTC, NTRK1, BRAF}, others) 9%, which are all mutually exclusive. These mutations are all effectors in the MAPK signaling pathway, the activation of which was confirmed through RNA sequencing, which showed robust induction of components of the MAPK transcriptional output (13), as well as by analysis of specific members of the phosphoproteome. \textit{BRAF} mutations are found in classical and tall cell variant PTCs, whereas \textit{RAS} mutations are highly prevalent in follicular variant PTCs, consistent with prior reports (14;15). The molecular taxonomy is different in advanced disease. Thus, although poorly differentiated (PDTC) and anaplastic thyroid cancers (ATC) also frequently harbor \textit{BRAF} mutations, they are comparatively enriched for \textit{RAS} mutations: 23-44% in PDTC (16;17) and 22% in ATC (18).

There is ample preclinical evidence that thyroid cancers retain dependency for viability on the constitutive activity of the oncogenic driver responsible for tumor development. This has been established in mouse models of \textit{Braf}-mutant papillary thyroid cancers (19;20), and is consistent with a paradigm that is now well accepted in oncology. Therapeutic targeting of oncoproteins such as BCR-ABL in chronic myelogenous leukemia (21;22), KIT in gastrointestinal stromal tumors (23), mutant EGFR in non-small cell lung cancer (24), and \textit{BRAF} in metastatic
ENHANCING EFFECTIVENESS OF RADIOIODINE THERAPY BY INHIBITION OF MAPK SIGNALING:

The natural history of metastatic thyroid cancer is often marked by a prolonged period of asymptomatic insidious disease progression. The only treatment option available to these patients in the past was radioiodine, which was often given repeatedly despite lack of evidence for benefit, as many patients with metastatic thyroid cancer have tumors that do not incorporate iodine efficiently. This is associated with worse prognosis: i.e. the 10-year survival of patients with metastatic thyroid cancer that retains RAI avidity is ~60%, whereas it is only 10% if the metastases are refractory to RAI therapy (27). Oncogenic activation of MAPK signaling in thyroid cells leads to loss of expression of genes required for thyroid hormone biosynthesis, including the sodium iodide transporter (NIS) and thyroid peroxidase (TPO) (28;29). The activating BRAFV600E mutation is the most frequent genetic alteration in PTC (30-32) and confers a comparatively worse prognosis (30;33-36). Tumors with BRAF mutation have lower expression of NIS (37), which likely explains the clinical observation that BRAF mutant PTCs are often particularly resistant to RAI therapy.

Mouse models of thyroid cancer driven by oncogenic BRAF develop tumors that recapitulate the observations in patients, in that they lose the ability to concentrate radioiodine. Moreover, when the activation of BRAF is switched off genetically, or its downstream signaling is targeted with small molecule kinase inhibitors of RAF or MEK, the tumors regain the ability to trap radioiodine (19). The experiments performed in mice provided the rationale for a pilot clinical trial in which patients with RAI-refractory metastatic thyroid cancer were treated with the MEK inhibitor selumetinib (AZD6244; AstraZeneca), in an attempt to restore RAI responsiveness. Briefly, patients known to have RAI-refractory distant metastatic disease underwent 124I PET scans after stimulation with recombinant TSH before and again after receiving a 4-week course of selumetinib. 124I is a positron-emitting isotope, which allows precise quantification of uptake in the metastatic lesions. Altogether, 12 of the 20 evaluable patients had marked increased uptake on the 124I-PET scans after treatment with the drug. Eight of these patients (8/20; 40%) had lesional uptakes that by dosimetry predicted that an effective dose of >2,000 cGy of 131I could be delivered, and they were therefore treated with a therapeutic dose of 131I. In these patients, the metastatic tumors decreased in size and the thyroglobulin levels, which serve as an effective biomarker for the disease, decreased dramatically. The responses were particularly striking in patients whose tumors harbored RAS mutations, whereas patients with BRAF-mutant disease had a more attenuated response (38). As discussed below, elucidation of the mechanisms accounting for the relative refractoriness of BRAF-mutant thyroid cancers to MEK and RAF kinase inhibitors provide promising strategies to improve on these outcomes.

OVERCOMING ADAPTIVE RESISTANCE TO RAF AND MEK INHIBITORS IN BRAF-MUTANT THYROID CANCERS:

By contrast to the high response rate seen in patients with metastatic melanomas (25;26), the RAF kinase inhibitor vemurafenib has limited efficacy as a single agent in patients with BRAF-mutant colorectal cancers (39). A clinical trial evaluating vemurafenib in BRAF-mutant thyroid cancer patients was recently completed, and presented in a late-breaking abstract by Marcia Brose at the European Cancer Organization’s (ECCO) 2013 European Cancer Congress. A 35% response rate was seen in patients not previously treated with multikinase inhibitors. Although encouraging, this rate of response is of a lesser magnitude than what has been reported for patients with metastatic melanoma. Hence, although cancers of these three
lineages (i.e. colon, melanoma and thyroid) harbor the same genetic driver, they differ in their response to a selective and highly effective inhibitor of mutant BRAF kinase.

Activation of MAPK signaling in non-transformed cells occurs in response to growth factors, cytokines and stress signals. BRAF-transformed cells hyperactivate MAPK independently of upstream inputs, to which cells become unresponsive through engagement of ERK-dependent negative feedback loops (40). Accordingly, RAS-GTP levels are depleted in BRAF-mutant tumor cells regardless of their cell type, and the activation state of receptor tyrosine kinases (RTKs) is low. When exposed to RAF or MEK inhibitors the signaling network of BRAF-mutant melanoma and thyroid cancer cells adapts by relaxing the repression of upstream signaling inputs, although they do so to a different extent depending in large part on the cell type. By contrast to melanomas, thyroid cells show rapid and robust increases in RAS-GTP shortly after exposure to RAF kinase inhibitors, due in large part to activation of HER3/HER2 signaling. This is caused by induction of HER3 and HER2 transcription, through decreased HER3 and HER2 promoter occupancy by the transcriptional repressors CtBP1 and 2. As BRAF-mutant thyroid cancer also constitutively secrete the HER3 ligand neuregulin-1, they are primed to reactivate signaling, which dampens responses to inhibition of mutant BRAF (41). The HER family kinase inhibitor lapatinib prevents MAPK rebound and sensitizes BRAF-mutant thyroid cancer cells to RAF or MEK inhibitors. This provides a rationale for combining inhibitors of the ERK pathway with inhibitors of feedback-reactivated HER signaling in this disease, and a phase I clinical trial with a combination of dabrafenib and lapatinib is currently in progress to test this hypothesis in patients.

Interestingly, patients with BRAF-mutant colorectal cancer are mostly unresponsive to the RAF inhibitor vemurafenib. The mechanisms of adaptive resistance to RAF kinase inhibitors in colorectal cancers is similar, but distinct, from that of thyroid cancer cells. It has recently been ascribed to activation of epidermal growth factor receptor (EGFR) signaling (42;43), due to feedback-induced relaxation of the activity of CDC25C, a putative EGFR phosphatase (42). Hence, although RTK induction may be ubiquitous in response to MAPK pathway inhibition, the determinants of adaptive resistance vary between cancer types. This is due to preferential upregulation of specific RTKs in different cancer cell lineages, and is also likely to be critically dependent on the abundance of their respective ligands in the cancer microenvironment, either through autocrine production or from other sources.

The reactivation of MAPK through RTKs or through derepression of wild-type RAS activity in response to RAF kinase inhibitors converges through MEK. Hence, combined therapy with RAF and MEK kinase inhibitors is a rational approach to overcome adaptive resistance. A combination of dabrafenib and trametinib was recently granted accelerated approval by the FDA for patients with metastatic BRAFV600E melanoma (44), and will soon be tested in patients with BRAF-mutant thyroid cancer.

ACQUIRED RESISTANCE TO RAF OR MEK INHIBITORS:

Patients whose cancers respond initially to inhibitors of the oncogenic driver of the disease (e.g. BRAFV600E melanoma to vemurafenib, EGFR-mutant non-small cell lung cancer to gefitinib) often find that these responses are not durable because of emergence of acquired resistance. About 50% of patients with EGFR-mutant lung adenocarcinomas that develop resistance to gefitinib or erlotinib acquire a second site mutation in EGFR that substitutes methionine for threonine at position 790 (T790M), the so-called “gatekeeper residue”, and which renders the receptor unresponsive to first line EGFR inhibitors. This paradigm, which recapitulates mechanisms of resistance to other selective kinase inhibitors (e.g. to imatinib in BCR-ABL mutant CML and KIT mutant GIST), provides further proof for the dependence of the tumor clone on the activity of the oncogenic driver for viability. In the case of BRAF-mutant cancers,
the largest experience so far is in patients with metastatic melanomas treated with vemurafenib. Acquired resistance occurs through selection of resistant clones that harbor activating mutations of RAS or MEK1, or that express a splice variant of BRAF, p61BRAF(V600E), which lacks exons 4–8, a region that encompasses the RAS-binding domain, and which shows enhanced dimerization that confers resistance to the inhibitor (45). There is no information so far on acquired resistance in the context of BRAF-mutant thyroid cancers. In those tumors that show a primary response to RAF inhibitors, resistance is likely to occur sooner or later. If and when they do, it will be important to define the mechanisms of resistance in biopsy material of lesions that progress while on therapy, since these will guide the design of combination therapies that may induce more sustained clinical responses.

A commonly stated concern is whether tumors that have a severely disrupted genome will still respond to selective therapies directed against a single oncoprotein. Patients with BRAF-mutant metastatic melanoma harbor lesions with a very high overall burden of mutations (46), and yet, as mentioned, have remarkable responses to RAF kinase inhibitors. A recent case report documented striking regression of the disease following treatment of a patient with BRAF-mutant anaplastic thyroid cancer with lung metastases with vemurafenib (47), suggesting that even this extraordinarily virulent malignancy may remain dependent for survival on the primary oncogenic driver.

TARGETING OTHER ONCOGENIC DRIVERS OF THYROID CANCER:
Most advanced cases of thyroid cancer harbor mutations of either BRAF or RAS. However, a significant fraction of metastatic thyroid cancers are driven by other mutant oncoproteins. Many of these, such as rearrangements of RET, ALK, NRTK1 or 3 are potentially drug-treatable. Despite their relative rarity, new approaches to trial design should now enable investigation of whether kinase inhibitors with activity against these mutant fusion oncoproteins provide clinical benefit. Other than for phase I studies, the conventional design of experimental therapeutic trials in oncology have been almost entirely disease-focused. Based on the strong evidence supporting the value of targeted therapies, trials can now be designed against mutant oncoproteins across disease types. This approach, which has been termed “basket trial”, enrolls patients with cancers harboring mutations of a specific oncoprotein on a treatment protocol with a drug that targets its activity, with each “basket” corresponding to a particular disease type (48). For instance, for a hypothetical basket trial of a drug targeting FGFR fusions, one basket could be for patients with bladder cancer, another for lung, another for thyroid, etc. A relatively small number of patients can be enrolled in each basket, allowing for simultaneous investigation of the role of the driver in different lineages. If activity is seen in one or more particular entity, that cohort can be expanded to allow for more meaningful conclusions. It is now conceivable that regulatory agencies may consider accelerated approval for drugs tested in this manner if the rationale is compelling, the results are clear and there is an unmet need.

CANCER IMMUNOTHERAPIES:
Rationally designed strategies targeting oncogenes and/or their signaling cascades are clinically effective, but the almost inevitable development of either adaptive and/or acquired resistance has precluded durable tumor responses in most patients. A new generation of immunotherapies has emerged that target negative regulatory receptors on T cells. Ipilimumab, the prototypic monoclonal antibody of this class, blocks the cytotoxic T cell receptor-4 (CTLA-4) on activated T cells (49). CTLA-4 is latently expressed on activated T cells during acute inflammation. Engagement of CTLA-4 by its ligand B7, expressed primarily on antigen presenting cells, suppresses T cell proliferation and helps restore tissue homeostasis. The clinical efficacy of targeting CTLA-4 in patients with metastatic melanoma was established in a milestone phase III clinical trial in which ipilimumab improved the overall survival of patients from 6.4 months to 10
months. Several patients exhibited complete responses that lasted for years. The results of this study led to the FDA approval of ipilimumab for patients with metastatic melanoma in 2011. A second emerging immunoregulatory target is the programmed cell death-1 (PD-1) receptor, which is expressed on hematopoietic cells, including T cells (50). The ligands for the receptor, PD-L1 and PD-L2, are expressed by antigen presenting cells, inflamed tissues, as well as by tumor cells, including thyroid cancers (51). Binding of PD-1 by PD-L1 or PD-L2 negatively regulates activated cytotoxic T cell functions and provides an additional mechanism by which tumors escape anti-tumor immunity. PD-1 blocking antibodies are showing remarkably durable responses in a number of tumor types, including lung, prostate, melanoma and others. The possible cooperation between these dual negative T cell checkpoint regulators on the adaptive immune response is also currently being explored in clinical trials with combined anti-CTLA-4 and anti-PD-1 blockade in patients with metastatic melanoma. These approaches have not yet been tested in thyroid cancer, but there is reason to believe that they may hold significant potential, since anti-thyroid autoimmune responses were noted in patients with metastatic melanoma treated with ipilimumab (52). Based on these and other recent breakthroughs in cancer immunotherapy (53) clinical trials will need to re-evaluate approaches that are based solely on the genetic characteristics of the tumors, and consider the place that each strategy, alone or in combination, may have in the treatment of different tumor types, including thyroid cancer.

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